University of Alberta

Potential for hybridization between native and non-native poplars in central Alberta

By

Arlene Huybregts

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in

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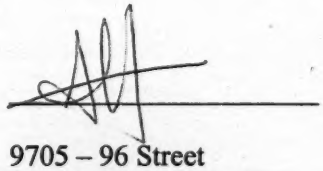

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled *Potential for hybridization between native and non-native poplars in central Alberta* submitted by Arlene Antonia Huybregts in Partial fulfillment of the requirements for the degree of Master of Science in Forest Biology and Management.

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ABSTRACT

In central Alberta, phenological flowering data were collected and graphed for forty clones of native balsam poplar, twenty clones of native aspen, and thirteen non-native poplar clones (seven hybrid poplar clones and six *P. davidiana* clones, an exotic pure species from China) growing in the same region. Based on the overlapping flowering patterns, the potential for hybridization between some native and non-native poplars exists, and then only if all other environmental and genetic compatibilities are met.

The next step was to determine if native and non-native poplars are genetically compatible by completing a greenhouse breeding experiment using branch material from native balsam and hybrid poplar clones. Seeds were collected, cleaned and tested for their viability by determining the percentage of normal and abnormal seedlings, and the percentage of dead seeds. The development of normal seedlings from some of the controlled crosses suggests that under the right environmental conditions hybrid seed can be produced from both female balsam and female hybrid poplars.

Consequently, the next step was to determine whether hybrid seed is being produced naturally by assessing leaf morphology (six variables used) of progeny from open-pollinated seed collected from both female balsam and hybrid poplar clones flowering adjacent to either native or hybrid male poplar clones. Early leaves were collected and measured from one-year-old plants and were compared with the leaf morphology from the controlled crosses (of the same age) derived from the breeding experiment using principal component and discriminate analysis. The fact that hybrid seeds were collected from female hybrid poplar clones Walker and Russian (from various locations) suggests that hybridization is occurring between native or hybrid male poplars and female hybrid poplars.

Based on the overlapping flowering patterns and the assessment of normal seedlings from both the controlled and the open-pollinated crosses the assumption is that the potential for hybridization between native and non-native poplars exists. However, there are other factors that may contribute to the potential for hybridization and may need to be considered when making management decisions on the installation of non-native

poplar plantations to help minimize the potential risk of gene flow into our native populations such as the relative abundance of native pollen, the relative distance between native populations and hybrid poplar plantations, and the selection of non-native poplars with earlier or later flowering patterns than native populations (to name just a few).

DEDICATION

I dedicate this thesis to my parents, Keith and Toni Huybregts, for their love and support in all of my endeavors, and to my partner, Rick Brzezowski, for his tremendous support and positive energy, which helped me to stay focused and complete this thesis after our baby girl, Lauren, was born.

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(over two winters) to designing and constructing the styro-boxes used in the breeding experiment. Besides offering physical 'man power', Rick offered emotional support. Essentially, Rick has been my 'rock' these past couple of years since we had our baby! Many hours were spent working on this project during evenings and weekends, which was time away from our family. Rick, I thank you for your unconditional love and unending support: your smile, patience, and encouragement have given me the strength to stay positive and focused to complete this thesis.

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CHAPTER ONE: General Introduction

1.1. *Introduction*

In the genus *Populus (P)* there are six taxonomic sections and nearly 31 different species (Figure 1-1) (Eckenwalder 1977; Dickmann and Stuart 1983), which are distributed throughout Eurasia and North America (Zsuffa 1975), and are commonly referred to as 'poplar'. The primary basis for identifying poplar species into distinct taxonomic sections is the morphological reproductive structures of male and female flowers (Eckenwalder 1996). The more general defining feature of poplars is their ability to reproduce clonally (asexually or vegetative) through the development of stump sprouts (all taxonomic sections), root suckers (all taxonomic sections), soft and hardwood cuttings (sections Aigeiros and Tacamahaca, poor in sections Populus, Leucoides, and Turanga), or buried branches (section Aigeiros) (Zsuffa 1975; Dickmann and Stuart 1983; Peterson and Peterson 1992; Eckenwalder 1996). A collection of root suckers or stem cuttings originating from a genetic source is referred to as a clone, where each individual stem is called a ramet (Dickmann and Stuart 1983). The ease of propagating stem cuttings has led to the process of cross breeding poplars for the production of hybrid poplars.

1.1.1. *Poplar breeding*

For the past 50-years, the study of intra- and inter-sectional hybridization of poplar species has been studied extensively and continues to be of interest among researchers and plant breeders. This interest is directly related to the opportunity to select superior individuals expressing hybrid vigor or other traits of interest for breeding material, followed by the ease of vegetative propagation to produce a large number of ramets in a relatively short period of time. A breeder's ability to produce a large number of ramets could ultimately benefit forestry operations by increasing productivity, improving wood quality, and decreasing rotation age (Peterson and Peterson 1992).

The expression of hybrid vigor is defined as the growth of any trait expressed in hybrid progeny, which surpasses the average growth of both parents or over that of the superior parent (Wright 1976). Hybrid vigor in natural populations is likely to exist, at least in some cases, between two closely-related species (same taxonomic section);

however, this is difficult to prove (Dickmann and Stuart 1983; Louis Zsuffa, Pers. Comm.). This unique hybridization phenomenon is believed to be more commonly found in artificially-produced hybrids (both intra- and inter-sectional crosses), as crosses are generally made with poplar material collected from distinctly separate geographical locations and adapted to different ecological environments (Zsuffa 1975; Stettler et al. 1996). The expression of hybrid vigor is believed to exist primarily in the first generation (F_1), decreasing with each succeeding generation (Zsuffa 1975; Wright 1976; Wu et al. 1992; Li and Wu 1996). Although hybrid vigor is widely recognized, it is not clearly understood (Hinckley et al. 1989; Li and Wu 1996; Li et al. 1998).

1.1.2. *Shelterbelt program*

In 1950, the Prairie Farm Rehabilitation Administration (PFRA) in Indian Head, Saskatchewan selected approximately eleven different hybrids (from sections Tacamahaca and Aigeiros) for their rapid growth rates and resistance to disease and insects, to be used in implementing their shelterbelt program. Through this program, hybrid poplar clones were propagated and distributed to private, agricultural landowners throughout the prairie provinces for the development of windbreaks (Brendon Casement and John Sharpe, Pers. Comm.), and subsequently, the use of hybrid poplars have been farmers' number one choice for the development of windbreaks (Stettler et al. 1996).

1.1.3. *Poplar farming*

More recently, the breeding of poplars is of increasing interest to the Canadian forest industry, even though as recently as 20-years ago, native poplar trees were considered a 'weed tree' to be removed and discarded from harvested areas. With the introduction of hardwood pulp mill plants, oriented strand board plants, and wafer-board plants, the utilization of poplars has dramatically increased since 1980 (Youngquist and Spelter 1990; Peterson and Peterson 1992). The use of hybrid poplar trees is also of special interest to the oil and gas industry because of the success of establishing hybrid poplar trees, particularly the hybrid clone Northwest (research study sites in northern Alberta), on sites disturbed through oil extraction (Barb Thomas, Pers. Comm.). In response to the increased utilization of poplars, the establishment of intensively-managed hybrid poplar plantations (poplar farming) has risen in both Canada and in the United States, helping to meet the increasing global demand for wood fiber. In Alberta, the

interest in poplar farming by the forest industry is escalating as hybrid poplar plantations are beginning to be installed by companies, such as Alberta-Pacific Forest Industries Inc. (Al-Pac), Daishowa-Marubeni, Millar Western Forest Products, and Weyerhaeuser Canada. To illustrate the extent to which hybrid poplars are used in Alberta, approximately ten non-native poplar species have been used to produce approximately 21 different hybrid poplar clones, which are currently located in either shelterbelts or on private poplar farms in Alberta (Figure 1-1).

Figure 1-1. Five taxonomic sections in the genus *Populus,* species within each section (closely related and hybridize freely), and the crossability of *Populus* species between taxonomic sections. Species used to develop various hybrid poplar clones found in central Alberta (circled), except for *P. davidiana* (a pure species from China).

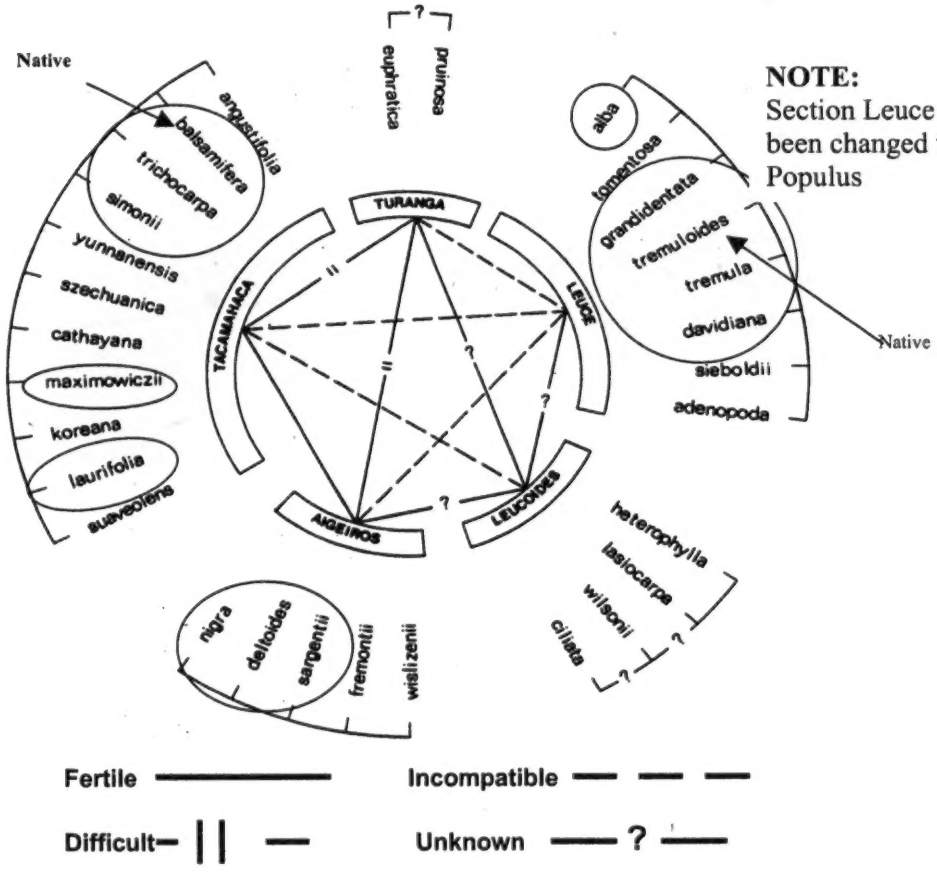


Figure adapted from Zsuffa (1975). This figure does not include the taxonomic section Abaso to accommodate *P. mexicana* (Eckenwalder 1977).

1.1.4. *Deployment of hybrid poplars*

In Alberta, the use of hybrid poplars is restricted to private land due to Provincial Government regulations (currently under re-development), which were put in place to avoid potential ecological risks such as hybridization followed by introgression with native poplars. Hybridization leading to introgression is a process where the genes of two or more sympatric species (native or introduced) are exchanged (Wright 1976). This can result in local populations of first generation (F_1) individuals and/or large populations comprised of many advanced generation hybrids and backcrosses to parental populations (original parent) (Eckenwalder 1984a). The concern is that through time the gene pool of native populations could be significantly altered, either positively or negatively, through hybridization with non-native species or hybrids (Anderson 1949; Eckenwalder 1984a). For example, if significant gene flow occurred between native and non-native (introduced) poplars, it could change the native population by producing progeny that grow faster, on the other hand, it may be deleterious to the native populations by decreasing growth rates due to high susceptibility to leaf rust, stem cankers, leaf and bud galls, or other pests (Henry and Barnes 1977). Ultimately the gene pool of native populations could be compromised. However, naturally occurring hybrid populations do occur when two or more *Populus* species from the same taxonomic section are sympatric, or when two or more *Populus* species from different sections are genetically compatible and sympatric.

1.1.5. *Natural hybridization*

Through fossil records it has been determined that species from sections Aigeiros and Tacamahaca have occupied distinctly different ecological habitats for at least twelve million years, and that hybrids have occurred during this time period (Eckenwalder 1984b; Henry and Barnes 1977). The hybrid *P. x jackii* (*P. deltoides x P. balsamifera*) is a naturally occurring hybrid found where populations of *P. deltoides* var. *occidentalis* (section Aigeiros) and *P. balsamifera* (section Tacahamaca) are sympatric, and in Alberta, a small natural hybrid swarm of *P. x jackii* has been reported near Drumheller (Eckenwalder 1984b). A clone of *P. x jackii* known as Northwest has been planted extensively throughout the United States and Canada, and was selected for its' hybrid vigor for PFRA's shelterbelt program (Eckenwalder 1984b).

In southern Alberta, *P. deltoides* and *P. angustifolia* James (section Tacamahaca) are at their most westerly native ranges, whereby both are sympatric and also within the range of native *P. balsamifera* (section Tacahamaca), and they have been reported to produce a tri-hybrid swarm known as *P. x acuminata* (Brayshaw 1965; Rood et al. 1985). However, in central and northern Alberta it is believed that there are no naturally occurring hybrid poplars, because native balsam poplar (*P. balsamifera*, section Tacamahaca) and native trembling aspen (*P. tremuloides*, section Populus, formerly called Leuce) do not hybridize naturally even though they are sympatric (Dickmann and Stuart 1983; Eckenwalder 1996). Although this cross has been reported successful under greenhouse conditions, it is very difficult to achieve (Zsuffa 1975; Willing and Pryor 1976; Dickmann and Stuart 1983).

Species found within the Populus section (formerly called Leuce) hybridize freely, but major reproductive barriers (biological reproductive structures) exist between the taxonomic section Populus and other sections (Figure 1-1) (Zsuffa 1975). However, the F_1 hybrids found in the shelterbelts of central and north-central Alberta might be biologically compatible with our native balsam poplars (section Tacamahaca), as their parentage is from sections Aigeiros and Tacamahaca. Therefore, the potential of hybridization and subsequent introgression may exist.

1.1.6. *Seed production and germination requirements*

Mature poplar trees are prolific seed producers. They can produce a million or more seeds (per tree) on an annual basis, exhibiting high germination rates (Peterson and Peterson 1992). Poplar seeds lack endosperm, therefore, seed viability is short, generally five days to two weeks, requiring a combination of environmental factors such as contact with warm, moist mineral soil, low evapotranspiration, no competition, and adequate surface moisture (Perala 1990; Peterson and Peterson 1992; Braatne et al. 1996). The establishment of poplar stands from seed has been reported in areas where the habitat has been disturbed due to man or natural forces such as in a variety of harvested or burned areas that were formerly occupied by other forest types (Barnes 1966; Navratil et al. 1989; Peterson and Peterson 1992; Kay 1993). However, widespread establishment of poplar stands from seed has probably not occurred since the last glacial and interglacial retreat, some 10,000 years ago in the western United States (Einspahr and Winton 1977)

and in Alberta (Cheliak and Dancik 1982). However, local populations have arisen, such as the establishment of aspen seedlings (ortets) in the Rocky Mountains following the 1988 fire in Yellowstone National Park (Kay 1993), and following the spring fire (near Legal) of 1999 in north-central Alberta (Ken Greenway, Pers. Comm.). Both of these burned areas were previously occupied by conifer tree species (Kay 1993; Ken Greenway, Pers. Comm.). However, seedling establishment in Yellowstone National Park was significantly reduced by ungulates (browsed developing shoots, which then died) (Kay 1993). In Alberta, successful seedling establishment following large fires has not been studied, but it is speculated that aggressive competing vegetation such as *Calamagrostis canadensis* is more likely to be the primary factor in preventing the survival of poplar seedlings rather than ungulates or drought (Ken Greenway, Pers. Comm.). Regardless of the amount of viable seed produced annually, existing poplar stands (as they are today) are predominately of clonal origin (Einspahr and Winton 1977, Cheliak and Dancik 1982; McDonough 1985; Jelinski and Cheliak 1992; Peterson and Peterson 1992).

In central and northern Alberta the landscape is a mosaic of trembling aspen and balsam poplar clones of various sizes. In west-central Alberta, poplar clones have been reported to range from 35 stems (0.03 ha) to 1002 stems (0.5 ha) (Peterson and Peterson 1992). Each clone is uniquely different in a number of morphological features such as stem form, and in phenotypic responses such as leaf size and shape, and the phenology of reproductive and vegetative buds (van Buijtenen et al. 1959; Barnes 1969).

1.2. THESIS OVERVIEW

1.2.1. *Objectives*

The overall objective of this research was to assess the potential for hybridization between native and non-native poplars in central and north-central Alberta. Chapter two is a determination of the phenological flowering patterns of native and non-native poplars, which is critical in predicting the potential for hybridization (Pregitzer and Barnes 1980). Phenology was measured to capture the initial timing of bud break, and the initial timing and duration (number of days) of pollen release and female receptivity.

In addition to understanding the phenology of flowering, determination of the genetic compatibility of native and non-native poplars was also undertaken through a greenhouse breeding experiment. Four mating combinations (native x native, native x hybrid, hybrid x native, and hybrid x hybrid), with various crosses within each mating combination, were conducted using forced branch material. Harvested seed was collected and tested for its' viability by determining the percentage of normal, abnormal and dead seeds. The breeding experiment and a discussion regarding the assumptions about the genetic compatibility between native and non-native poplars are presented in Chapter three. Chapter four is an investigation into whether hybridization is occurring between native and non-native poplars. Open-pollinated seeds (known mother and unknown fathers) were collected from native and hybrid female poplars adjacent to both native and hybrid male poplars. Seeds were sown in the greenhouse, and leaves were measured from each seedling to determine if putative hybrids are present by comparing their leaf morphology with that of the progeny of known parentage (both pure species and hybrid crosses) obtained from the greenhouse breeding experiment in Chapter three. Chapter five is a summary and synthesis of the material presented in chapters two to four.

The information gathered in this research is the first step to understanding the potential for hybridization between native and non-native poplars, and the factors to consider when installing hybrid poplar plantations, which could help minimize the potential ecological risks.

1.2.2. *Approach*

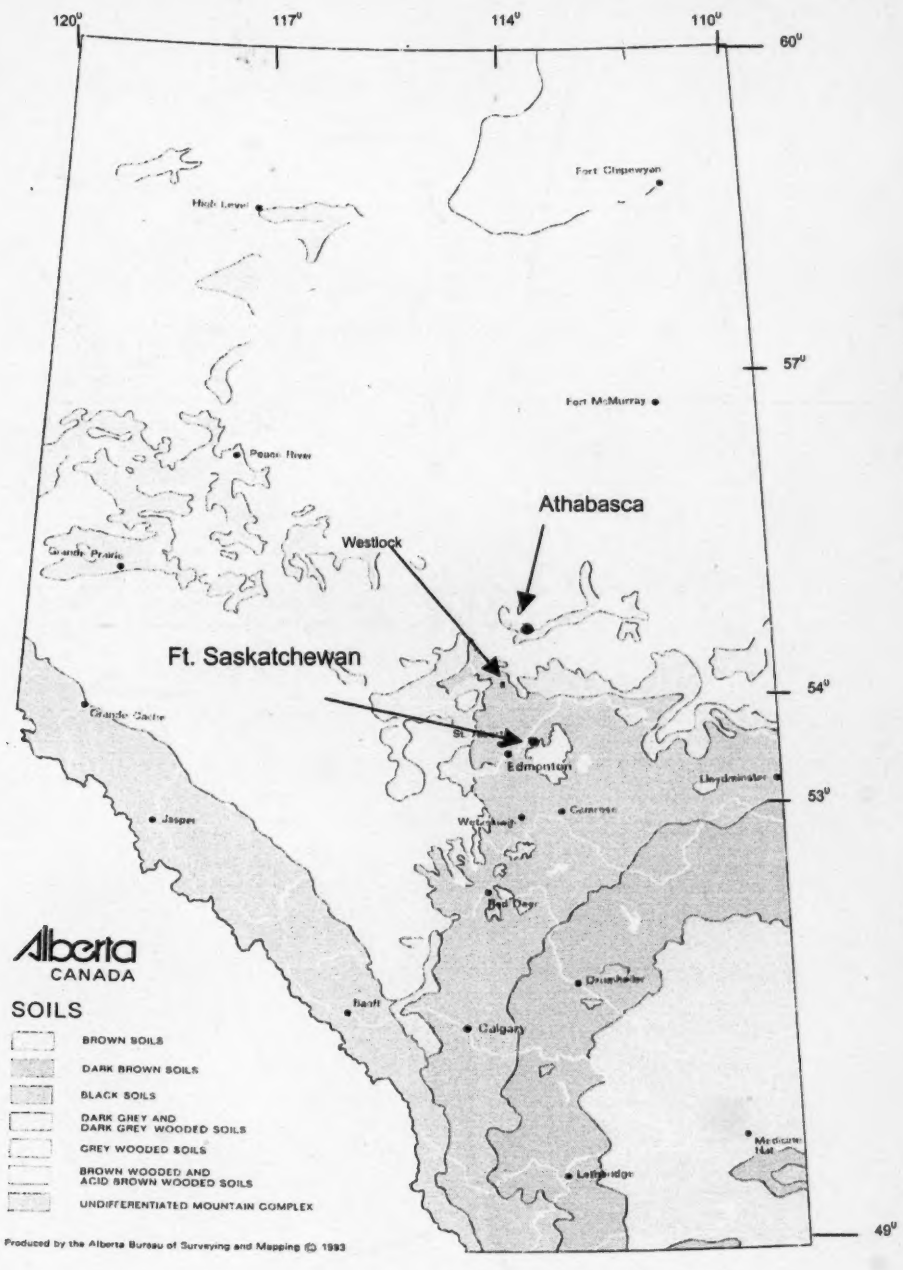
In order to conduct this research, information provided by Alberta-Pacific Forest Industries, Inc. (Al-Pac) was used. During the early 1990's, Al-Pac compiled a list of hybrid poplar clones on private land from the east-central region of Alberta from Edmonton (53.4^0N latitude (lat.), 113.6^0 longitude (long.)) north to Athabasca (54.6^0N lat., $113.^0$E long.), east to Lloydminister (53.2^0N lat., 110.1^0E long.) and west to Westlock (54^0N lat., 113.9^0E long.). In this region, information collected included legal land description, land-owner's name, type of hybrid poplar clone, planting year, number of trees planted, and gender. In 1999, this information was used to initially locate and observe the status of hybrid poplar clones (dead or alive), and to confirm their gender. Several discrepancies were found, and in some cases the original landowner was

unavailable to discuss the type of hybrid poplar clone planted (deceased or moved away). However, there were four hybrid poplar clones (Brooks 1 (also known as Griffen), Brooks 6, Russian and Northwest) planted extensively throughout the region, and therefore, these clones were used in this project.

1.2.3. *Study areas*

In January 2000, both Fort Saskatchewan (53.6^0N latitude, 113.4^0E longitude) and Athabasca (54.6^0N latitude, 113^0E longitude) were selected as study areas, based on the number of different hybrid poplar clones found within a relatively small geographical area (Figure 1-2). Additional considerations for selection included the proximity of hybrid poplar clones to native trees and accessibility (land owner's approval). In addition, hybrid poplar clones Tristis, Walker, Manitou, and the pure species *P. davidiana* (an exotic aspen species from China) were also used in this study, but they were located only in the Athabasca study area. In order to compare the flowering patterns between native and non-native poplars, both male and female native *P. tremuloides* and *P. balsamifera* clones were also selected.

Figure 1-2. Map of Alberta denoting the Fort Saskatchewan and Athabasca study areas.



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CHAPTER Two: Flowering Phenology

2.1. INTRODUCTION

2.1.1. *Background*

Poplars are typically dioecious, where trees bear either male or female flowers, unlike most temperate tree species, which are monoecious (male and female flowers borne on the same tree). However, the occurrence of hermaphroditism (presence of both male and female flowers on the same tree) has been reported in some *Populus* species (Santamour 1955; Pauley and Mennel 1957). The flowers of both male and female trees are borne on pendant racemes known as catkins (Zsuffa 1975; Eckenwalder 1996). In most *Populus* species male and female catkins are easily distinguished from each other during the early stages of flower development, as male catkins are reddish, and females are greenish in appearance (Braatne et al. 1996).

In natural stands, the average flowering age is between 15 and 20 years, with maximum flower production and seed set occurring between 50 and 70 years (Einspahr and Winton 1977). However, flower production and seed crops occur much earlier in poplar plantations, as seed set has been observed in three-year-old plantations of *P. tremuloides,* (Pauley 1950), *P. deltoides* (Cooper 1990), and in six-year-old plantations of *P. davidiana* (Pers. Observ.).

Reproductive (male and female flowers) buds are formed by the end of the growing season, and enter a state of dormancy during the fall. Male trees typically produce flowers on an annual basis, but the production of female flowers varies from year to year (Einspahr and Winton 1977). It is believed that the development of female flowers is associated with growth suppression due to moisture stress, which occurs during hot, dry summer conditions when flower buds are being developed (Cannell 1989). In the following spring, both male and female flowers emerge, prior to leaf expansion, in response to warming spring temperatures after a period of cold dormancy (Pauley 1950; Ahlgren 1957; Cannell 1989; Stanton and Villar 1996). The onset and duration of flowering is known to vary within and between poplar species (Farmer 1966; Perala 1990), and flowering is assumed to be under moderate to strong genetic control (heritable) (Farmer 1966; Cannell 1989).

Flowering data can be graphed and used to look at the potential for hybridization between two or more sympatric species (native or introduced) that are genetically compatible based on the degree of overlapping flowering patterns (Ronald et al. 1973; Pregitzer and Barnes 1980), as the timing of flower development is known to be one of the major reproductive barriers preventing gene flow (Wright 1976). This chapter reports on the timing of bud break and the timing and duration of initial pollen release and female receptivity of native (balsam and trembling aspen) and non-native (hybrid poplar and *P. davidiana* clones) poplars planted in the same area.

2.1.2. *Objective*

The goal of this study was to observe and record the phenological flowering patterns (timing and duration of pollen release and female receptivity) of native and non-native poplars, and to explore the extent to which flowering phenology can affect the likelihood for hybridization.

2.2. MATERIAL AND METHODS

2.2.1. *Selected material*

For each of the four hybrid clones found in the shelterbelts, two replicates (2 different locations) within each study area were monitored to determine if similar flowering patterns were expressed, except for the hybrid clone Brooks 6 in Athabasca, where only one replicate was available. In addition, the hybrid poplar clones Tristis (shelterbelt clone), Walker, Manitou and seven exotic clones of *P. davidiana* (Al-Pac mill site) were selected from the Athabasca study area (Table 2-1 and 2-2), and there was only one replicate (one location) for each of these clones, as they were only planted in one (known) location (see Appendix A for legal land description for each native and non-native clone).

To compare the flowering patterns of the non-native poplars (Table 2-1) with native poplar trees, I selected eighteen clones of balsam poplar (10 females and 8 males) and ten trembling aspen clones (5 males and 5 females) in Athabasca, and twenty clones of balsam poplar (10 males and 10 females) in Ft. Saskatchewan (Table 2-2 and 2-3). During the trees dormant phase, gender of individual trees can be identified using a high-powered hand lens to examine floral primordia by removing bud scales and floral bracts (Figure 2-1) (Kaul 1995). A pruning pole (5.5 meters) and a ladder were used to cut lateral branches with reproductive buds from each lower tree crown to determine gender.

Table 2-1. Non-native *Populus* trees: Hybrid poplar clone or exotic species name, parentage, parent origin and verification.

Clone or species name[1]	Parentage (female x male)	Parent origin	Verification[3]
Northwest (M)	*P. deltoides x P. balsamifera* Section: Aigeiros x Tacamahaca	North America x North America (NA)	Dickmann and Stuart (1983) and PFRA[3] (1999)
Russian (F) *P. x petrowskyana*	*P. laurifolia x P. nigra* Section: Tacamahaca x Aigeiros	Asia (Siberia) x Eurasia	Dickmann and Stuart (1983)
Brooks 1, 6 (M) and Walker (F)	*P. deltoides x P. x petrowskyana* Section: Aigeiros x [Tacamahaca x Aigeiros]	NA X [Asia (Siberia) x Eurasia]	Dickmann and Stuart (1983); PFRA
Manitou (M)	O.P. Walker[2] Section: [Aigeiros x [Tacamahaca x Aigeiros]] x unknown		PFRA
Tristis (M)	*P. tristis x P. balsamifera* Section: Tacamahaca	Central Asia (Himalaya) x NA	Dickmann and Stuart (1983)
P. davidiana 3 F & 4M	O.P. *P. davidiana* Section: Populus (formerly Leuce)	China	Al-Pac[4]

1. (M) = male clone, (F) = female clone.
2. O.P. = open pollinated (unknown male parentage e.g.. *P. deltoides* or *P. balsamifera*).
3. PFRA = Prairie Farm Rehabilitation Administration.
4. Al-Pac = Alberta Pacific Forest Industries Inc.

Table 2-2. Hybrid poplar clones, *P. balsamifera* (20 clones), *P. tremuloides* (10 clones) and *P. davidiana* (7 clones), gender (*), replicate, and age of hybrid poplar clones and location of trees in the Athabasca study area (*).

Hybrid[1], and species (and number of clones within a species)	**Gender[2]**		**Replicate[3]** and **age** (yrs)		**Location**	
	M	**F**	**1**	**2**	Athabasca	Al-Pac's mill
Brooks 1	*		15 yrs	25 yrs	*	
Brooks 6	*		15 yrs		*	
Northwest	*		15 yrs	10 yrs	*	
Russian		*	20 yrs	35 yrs	*	
Tristis	*		35 yrs		*	
Walker		*	10 yrs			*
Manitou	*		10 yrs			*
P. davidiana[4] (Dav)						
Dav 1 (#37)		*	6 yrs			*
Dav. 2 (#38)	*		6 yrs			*
Dav. 3 (#39)		*	6 yrs			*
Dav. 4 (#40)		*	6 yrs			*
Dav. 5 (#41)	*		6 yrs			*
Dav. 6 (#42)	*		6 yrs			*
Dav. 7 (#43)	*		6 yrs			*
P. tremuloides (Aw)						
Aw 1		*				*
Aw 2	*					*
Aw 3		*				*
Aw 4	*					*
Aw 5	*					*
Aw 6		*				*
Aw 7	*					*
Aw 8		*				*
Aw 9		*				*
Aw 10	*					*
P. balsamifera (Pb)					*	
Pb 1		*			*	
Pb 2	*				*	
Pb 3	*				*	
Pb 4	*				*	
Pb 5		*			*	
Pb 6		*			*	
Pb 7	*				*	
Pb 8		*			*	
Pb 9		*			*	
Pb 10		*			*	
Pb 11	*				*	
Pb 12	*				*	
Pb 13	*				*	
Pb 14		*			*	
Pb 15	*					*
Pb 16		*				*

Pb 17		*				*
Pb 18		*				*

1. Hybrid clone = see parentage in Table 2-1.
2. Gender M = male, F = female.
3. Replicate = only relevant to hybrid poplar clones. Age = not relevant to native *P. tremuloides* and *P. balsamifera.*
4. *P. davidiana* = Numbers 37 – 43 are numbers allocated to each seedlot and can be referenced in Figure 2-12.

Table 2-3. Hybrid poplar clones, *P. balsamifera* (20 clones), gender (*), replicate, and age of hybrid poplar clones in Ft. Saskatchewan.

Hybrids[1], and *P. balsamifera* (and 10 clones)	**Gender[2]**		**Replicate[3]** and **age** (yrs)	
	M	**F**	**1**	**2**
Brooks 1	*		8	30
Brooks 6	*		35	15
Northwest	*		30	35
Russian		*	30	20
P. balsamifera (Pb)				
Pb 1		*		
Pb 2	*			
Pb 3	*			
Pb 4	*			
Pb 5		*		
Pb 6		*		
Pb 7		*		
Pb 8		*		
Pb 9		*		
Pb 10	*			
Pb 11	*			
Pb 12	*			
Pb 13	*			
Pb 14		*		
Pb 15		*		
Pb 16	*			
Pb 17	*			
Pb 18		*		
Pb 19		*		
Pb 20	*			

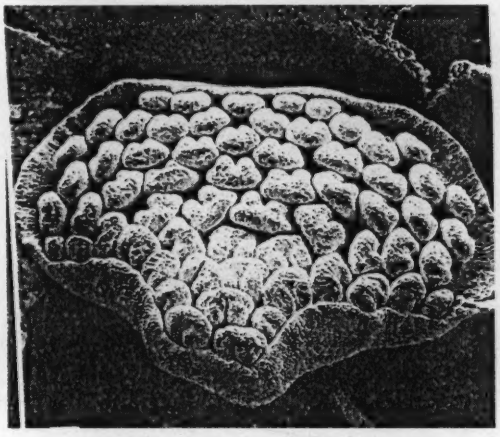
1. Hybrid clone = see parentage in Table 2-1.
2. Gender M = male, F = female.
3. Replicate = only relevant to hybrid poplar clones. Age = only relevant for hybrid poplar clones.

Figure 2-1. Diagnostic features of male and female flowers (observed through a hand lens, 35X) during the tree's dormant phase.

Male Flowers

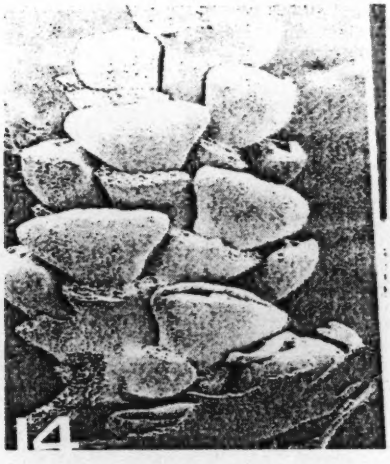
Bud scales removed showing bracts and anthers.

Perianth cup containing stamens

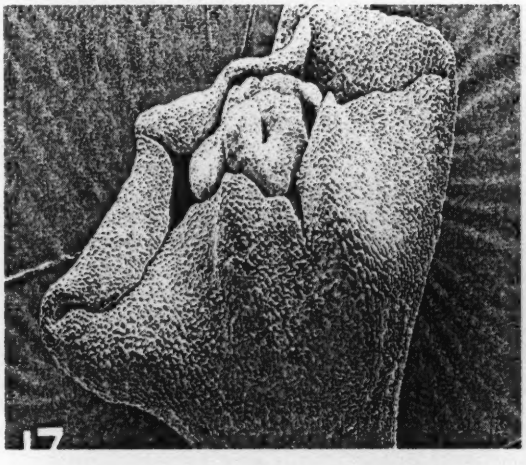


Female flowers

Bud scales and bracts removed showing advanced floral primordia



Stigmas can be observed inside the folded perianth.



Photos from Kaul 1995.

Typically, the identification of clones within a species is based on morphological and phenological characteristics such as bark, stem form (vanBuijtenen et al. 1959), leaf size and shape, and timing of leaf flush (Barnes 1969). However, both the Athabasca and Ft. Saskatchewan study regions are agricultural communities where large native poplar stands are not accessible by road because of the large areas of agricultural land. Native trees do exist as individuals along roads and fences, but it is difficult to determine intra-clonal variation based on morphological and phenological characteristics. Therefore, selected native trees were at least one kilometer apart to ensure each tree was from a different clone (Steneker 1973; Farmer and Reinhold 1986).

Two ramets from each clone (except for the seven clones of *P. davidiana*, which originated from seed) were selected, and the crowns of individual ramets were divided into three sections (lower, middle and upper). Since all selected trees were open grown with no competition for sunlight, crown aspect was not considered, as Pregitzer and Barnes (1980) assessed the flower patterns of native *Populus* species and observed no significant developmental pattern with respect to crown aspect (or within a crown).

2.2.2. *Approach*

Prior to collecting spring (2000) field data, a good understanding of how male and female flowers develop was necessary in order to accurately describe the timing of initial pollen release and female receptivity. Therefore, a basic assessment of flower development was conducted. In January 2000, branches from several male and female hybrid poplar clones were cut and placed in buckets of water to force flowers to develop using the 'cut branch technique' (Einspahr 1960). Flowers were monitored daily and photos taken throughout the development stages. Selected photos were then used to develop a sequential series of categories depicting a quantitative scoring system to apply during the collection of spring data. This scoring system was divided into four developmental stages and used to describe flower development in the lower, middle and upper tree crowns for individual ramets on each sample day (Figure 2-2).

Figure 2-2. Description and photos of the four developmental stages for both male and female poplar flowers.

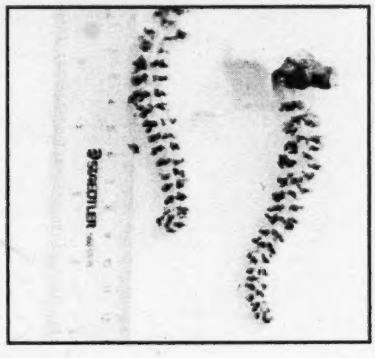
Stage I, bud break for male and female flowers: In the early spring (April) bud scales begin to separate, making room
for the arising flowers (male and female).

Male Flowers only –

Stage II, catkin elongation: Flowers emerge approximately 2cm from the bud sheath and exposed anthers appear dark red in color (Stanton and Villar 1996) with attached subtending brown bracts (Kaul 1995). As flower development progresses, rachis (main stem of catkin) elongates and droops, mature androecium (a group of stamens) become less crowded, and the subtending bracts fall off (Kaul 1995).

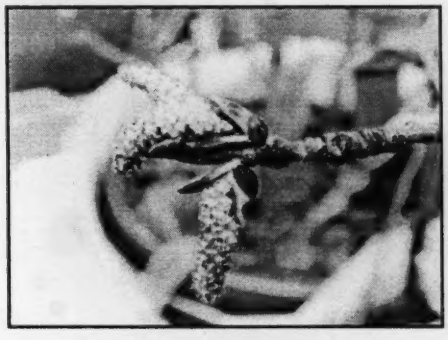
Stage III, pollen release: As anthers ripen, color changes from dark red to yellow (pollen release).

Stage IV, spent flowers: Depleted flowers appear dried or withered (dark brown in color), and abscission occurs.



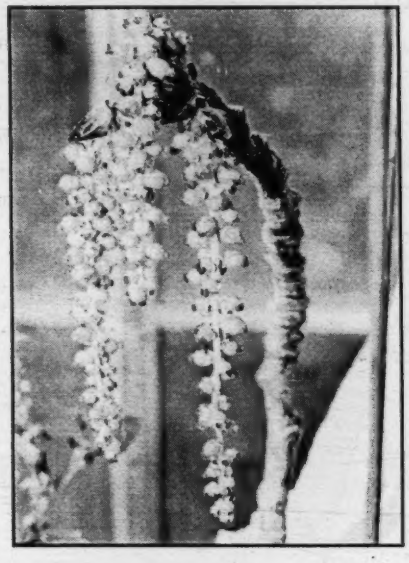
Female Flowers only –

Stage II, catkin elongation: Flowers emerge approximately 2cm from the bud-sheath and pistillate flowers are tightly closed, surrounded by white bracts. As flower development progresses, the rachis elongates and the subtending bracts expose the emerging stigmas (Kaul 1995). Stigmas vary in color from light green in sections Tacamahaca and Aigerios (Stanton and Villar 1996) to rose-colored in *P. tremuloides* (section Populus) (Einspahr and Winton 1977).



Stage III, female receptivity: Rachis continues to elongate, and female flowers are receptive when floral bracts have fallen, exposing light green (or reddish) stigmas and dark green ovaries beyond the perianth cup. Visually, catkins appear to look plumper (fuller), due to the exposed stigmas, and there is an apparent separation between stigmas, as the rachis elongates (Stanton and Villar 1996; Kaul 1995).

***Stage IV, developing seed pods*:** Within approximately 6 hours after pollination, ovules have swollen to fully occupy the perianth cup (Fechner 1972), stigmas wither, and rachis continues to elongate while green fruits enlarge quickly (Kaul 1995).



2.2.3. *Data Collection*

Flower development was monitored and scored for catkins within each crown section, using the categorical scoring system described above. In March 2000, trees were monitored weekly for bud break (stage I) with the aid of binoculars (8X42). After bud break, observations were taken every second day until catkins (flowers) started to elongate (stage II), when daily monitoring commenced. On each sample day, flowers within each crown section were categorized into one of the four stages of development. As flowers progressed into stage III (pollen release and female receptivity), a percentage value was given to each crown section, expressing the average number of flowers releasing pollen (based on color change from red to yellow) and receptivity of females (based on flower development described in stage IV), along with a written statement describing the changes.

To remove extraneous variables from the data and to ensure consistency, measurements were taken at approximately the same time during each sample day, and

only one field technician per study area was used. Random samples of male and female catkins were collected daily to confirm visual estimates of the first and last day of pollen release and female receptivity.

2.2.4. *Flower assessment*

A scatter diagram was used to show the development of flowers from stages I to IV within the crown of two ramets for each of the four hybrid poplar clones selected from the shelterbelts. Initially, stages I to IV were plotted for each crown section of an individual ramet (for each sample day), but the results showed little variation between the three crown sections and the two ramets (no data shown). Therefore, flower development within a crown (stages I to IV) was apparent by averaging the reproductive stage across the three crown sections of an individual ramet, and then averaging across both ramets for each sample day.

To assess intra-clonal variation between individual ramets of native balsam and hybrid poplars, a vertical bar graph was used to show the total amount (percentage) of pollen released and female receptivity (stage III) within a crown of an individual ramet, by averaging the amount of pollen released and female receptivity across the three crown sections of an individual ramet for both native balsam and hybrid poplar clones (for each sample day). The method outlined above was also used to observe variation between the two replicates of the four-hybrid poplar clones selected from the shelterbelts.

A horizontal line and bar graph was used to assess intra- and inter-clonal variation of *P. tremuloides, P. davidiana, P. balsamifera* and among the hybrid poplar clones. The horizontal lines represent anthesis (timing of flower development from stage I to stage IV), and the horizontal bars represent stage III (the timing and duration of pollen release and female receptivity).

To observe the flowering patterns of native (trembling aspen and balsam) and non-native (*P. davidiana*, and hybrid poplars) poplar clones, spline curves (Sigma Plot, Version 7.0) were used to examine the zone of flowering overlap by averaging the total amount of pollen released and female receptivity (stage III) across the three crown sections of individual ramets, across both ramets, and then across all clones (for each gender) of *P. balsamifera, P. tremuloides, P. davidiana* and the hybrid poplar clones.

2.3. RESULTS

2.3.1. *Visual observations*

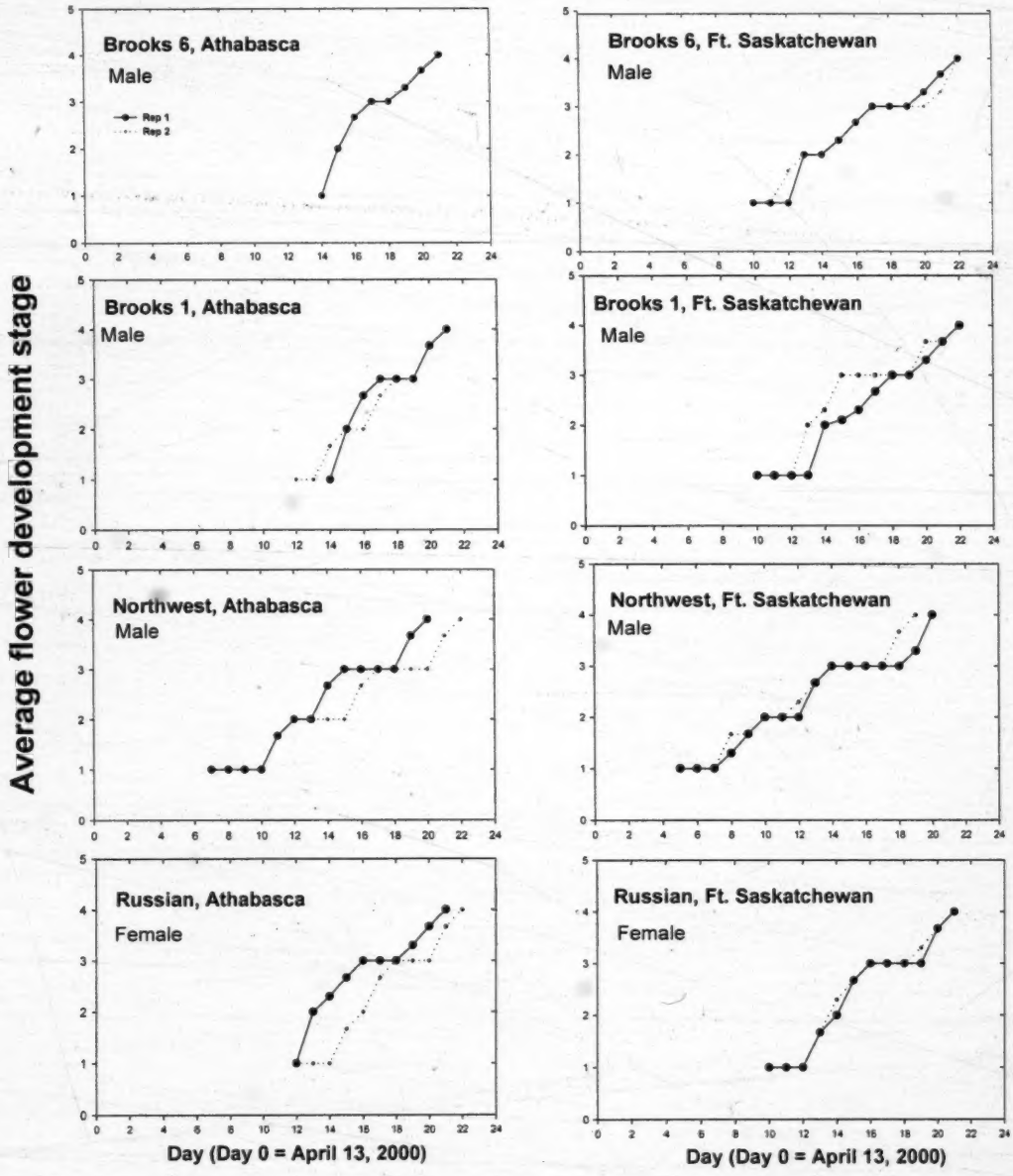
The technique was useful in determining the initial timing and duration of pollen release. The observation of male catkins changing color from red (stigmas) to yellow (pollen) was noticeable throughout the whole crown of an individual tree; therefore, daily samples were not necessary for capturing the initial timing of pollen release. However, sample collections were useful in capturing the final stages of pollen shed.

For female flowers, the technique used was not as useful in determining the initial timing of female receptivity, as female flowers did not have an obvious change in color compared to the male flowers. The development of female flowers from elongation (stage II) to receptivity (stage III) is subtle. Field technicians were able to distinguish between stage II and III by becoming aware of noticing that when the rachis elongates, individual flowers separate to make room for the emerging stigmas, which causes the catkins to look plumper (the initial stage of female receptivity - stage III).

Flower development of individual racemes (flowers) across the three crown sections of a ramet appeared to progress simultaneously from stage I (bud break) to stage II (catkin elongation). However, as flowers moved into stage III (pollen release and female receptivity) flower development among individual flowers (catkins) progressed discretely both within and between crown sections. During flower development, individual flowers within a crown section, and between the lower and the combined middle and upper crowns were typically one day (rarely two days) apart developmentally, as previously described by Pregitzer and Barnes (1980). This method of flower progression has been described as 'normal' for most forest tree species (Sarvis 1962).

Flower development within the crown of a ramet advanced from stage one to stage four continuously; however, flower development typically remained at stage I and III for several days, as individual flowers progressed from stage to stage discreetly. For the hybrid poplar clones located in shelterbelts, stage I appeared to last from two to four days and stage III from two to five days (depending on the clone) (Figure 2-3).

Figure 2-3. The average flower development stage (see Figure 2-2) by day, across three crown sections, and across two ramets for replicates 1 and 2 for Brooks 6, Brooks 1, Northwest, Russian in both Athabasca and Ft. Saskatchewan (See Material and Methods for description of stages).

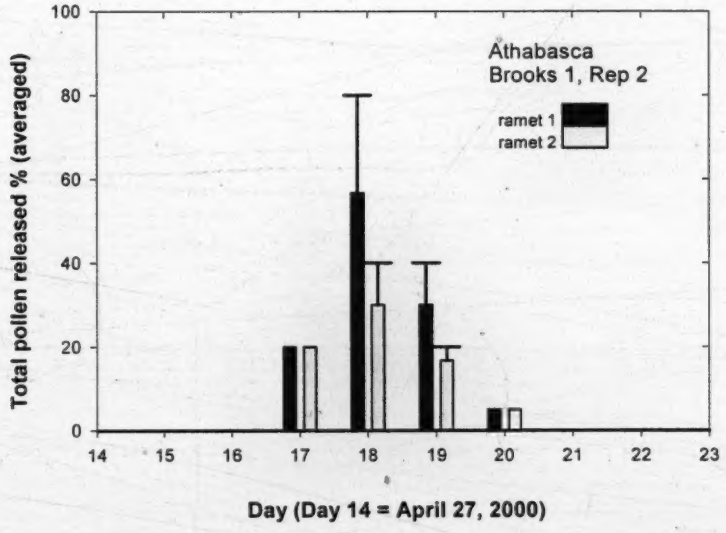


There were four clones in Athabasca that did not exhibit standard flower development: 1) one of four native balsam poplar clones (Pb4) developed normal flowers, but no pollen was released (no data collected), stigmas changed color from red to a dark purplish color and then appeared shriveled and dried up; 2) two female clones (native balsam clone #1 and Russian in rep2) produced receptive flowers, but no seed was produced. Receptive stigmas withered and racemes exhibited premature abscission by the end of the data collection period (2nd week of May, 2000); 3) one of seven *P. davidiana* (Dav. 41) clones exhibited frozen buds, as flowers did not emerge and were black in color (no data collected).

2.3.2. *Intra-clonal variation between ramets and replicates*

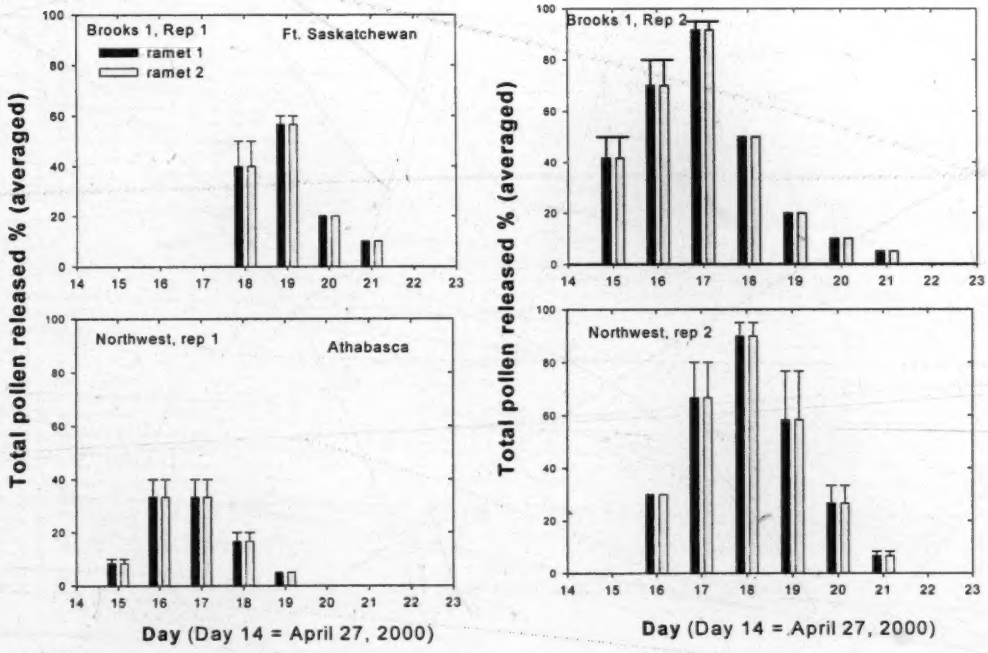
Ramets: During stage III, there was no observed variation in the amount of pollen released or female receptivity between ramets within a clone for both the native balsam and hybrid poplar clones (Appendix B), except for Brooks 1 (in Athabasca), where the total amount of pollen released from ramet 1 was approximately 50% more than ramet 2 (Figure 2-4). Although the two ramets were morphologically similar, they were planted in different years.

Figure 2-4. The average amount of pollen released across the three crown sections by ramet for Brooks 1 in Athabasca.



Replicates between the four-hybrid poplar clones located in shelterbelts: During stage III, replicates of the four hybrid poplar clones varied both within and among study areas. It appeared that the initial timing of pollen release and female receptivity (stage III) differed between replicates (rep) within a study area by one or two days (see Appendix B), except for Brooks 1 (in Ft. Saskatchewan) and Northwest (in Athabasca). Brooks 1 first released pollen on day 18 (May 1) for the ramets located in rep 1, and on day 15 (April 28) for ramets located in rep 2, a difference of three days (Figure 2-5). In addition, the total amount of pollen released by the ramets in rep 1 appeared to be approximately 50% less than the ramets in rep 2 (Table 2-5). The ramets of Northwest located in rep 1 also appeared to release approximately 50% less pollen than the ramets located in rep 2 (Figure 2-5).

Figure 2-5. Variation in the timing of initial pollen release and the total amount of pollen released between replicates of Brooks 1 (Ft. Saskatchewan) and Northwest (Athabasca).



2.3.3. *Verification:*

Leaf tissue was collected from all native balsam and hybrid poplar clones used in this study for the extraction of DNA (not part of this study). Results from the DNA analysis, using 13 Simple Sequence Repeat (SSR) markers showed that the ramets of each hybrid clone located in shelterbelts were accurately labeled, except for one clone, Brooks 1. The DNA fingerprint of replicate 1 of Brooks 1 in Ft. Saskatchewan (data graphed in Figure 2-5) indicate a mislabeling, as all 12 of the optimized markers were identical to the DNA fingerprints of the hybrid clone Brooks 6. Whereas the ramets of Brooks 1 in replicate 2 (Figure 2-4) in Ft. Saskatchewan appear to be genetically similar in 12 of the 13 optimized markers, probably indicating that both ramets are Brooks 1. According to the Research Associate doing this work, this genetic variation observed at one locus could be due to clonal variation, as each time a hybrid (or pure species) is propagated, localized mutations can occur, but they may not be expressed (Dr. Muhamad Rahman, Pers. Comm.; Dickmann and Isebrands 2001).

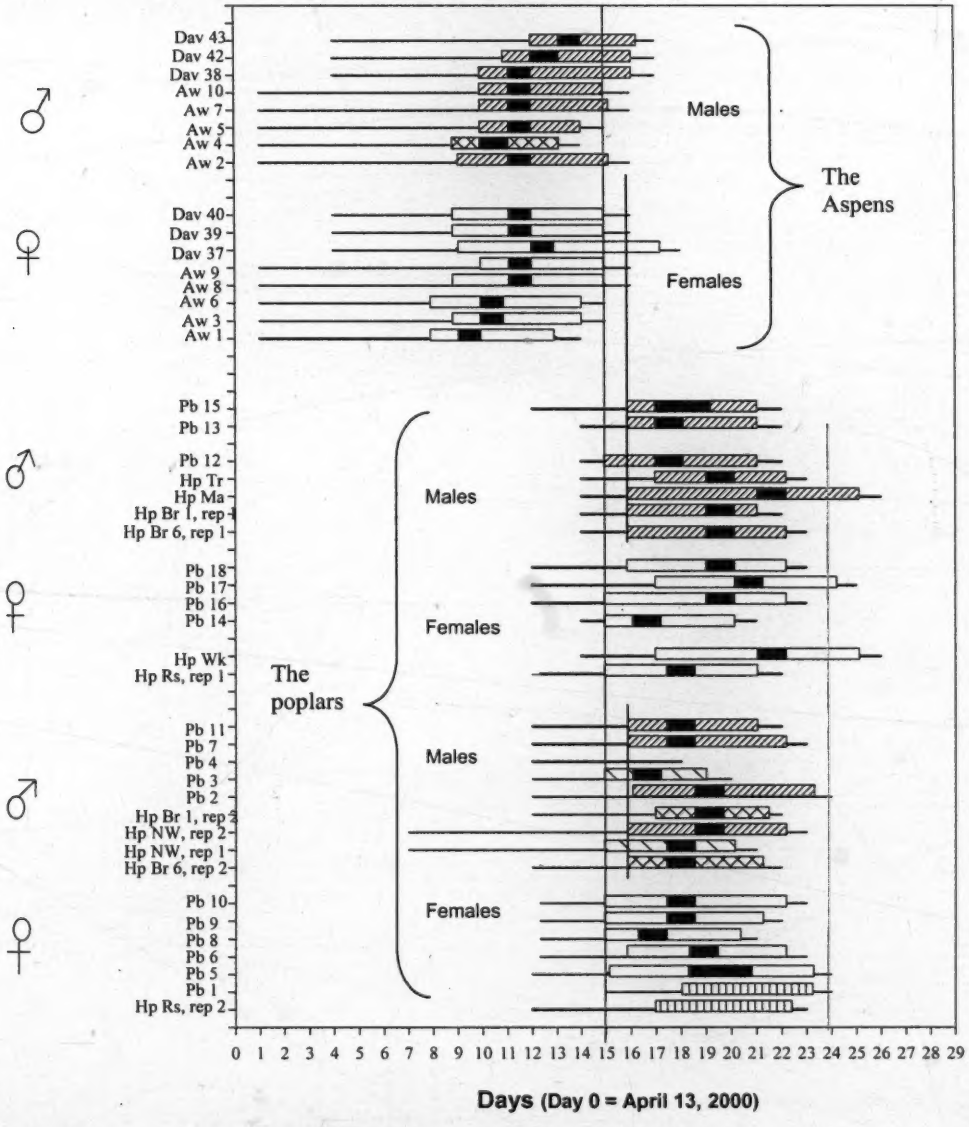
From the DNA analysis of the ramets for replicates 1 and 2 of Northwest (Table 2-5), all trees appear to be genetically the same.

2.3.4. *Inter-clonal variation within and between native and non-native poplars*

The aspens (P. tremuloides and P. davidiana) in Athabasca: It was observed that floral bud break of male and female clones of *P. tremuloides* occurred on day 1 (April 14) followed by *P. davidiana* clones on day 4 (April 17) (Figure 2-6). The flowering pattern (timing and duration of fertility) of female *P. davidiana* appeared to overlap with the flowering pattern of native male *P. tremuloides.* The timing of peak receptivity for two of the three female *P. davidiana* clones (dav 39, 40) overlaped with the timing of peak pollen shed for four of the five clones of *P. tremuloides* (Aw 2, 5, 7, 10) (Figure 2-6 and 2-7A). Similarly, the flowering pattern of native female *P. tremuloide*s and male *P. davidiana* also appears to overlap, although not to the same degree as described above (Figure 2-7B). The timing of peak receptivity for only two of the five *P. tremuloides* clones (Aw 8, 9) overlapped with the timing of peak pollen shed for one of the three male clones of *P. davidiana* (Dav 38, 42, 43) (Figure 2-6 and 2-7B). The other three female clones of *P. tremuloides* (Aw 1, 6, 3) appeared to reach peak receptivity between one and

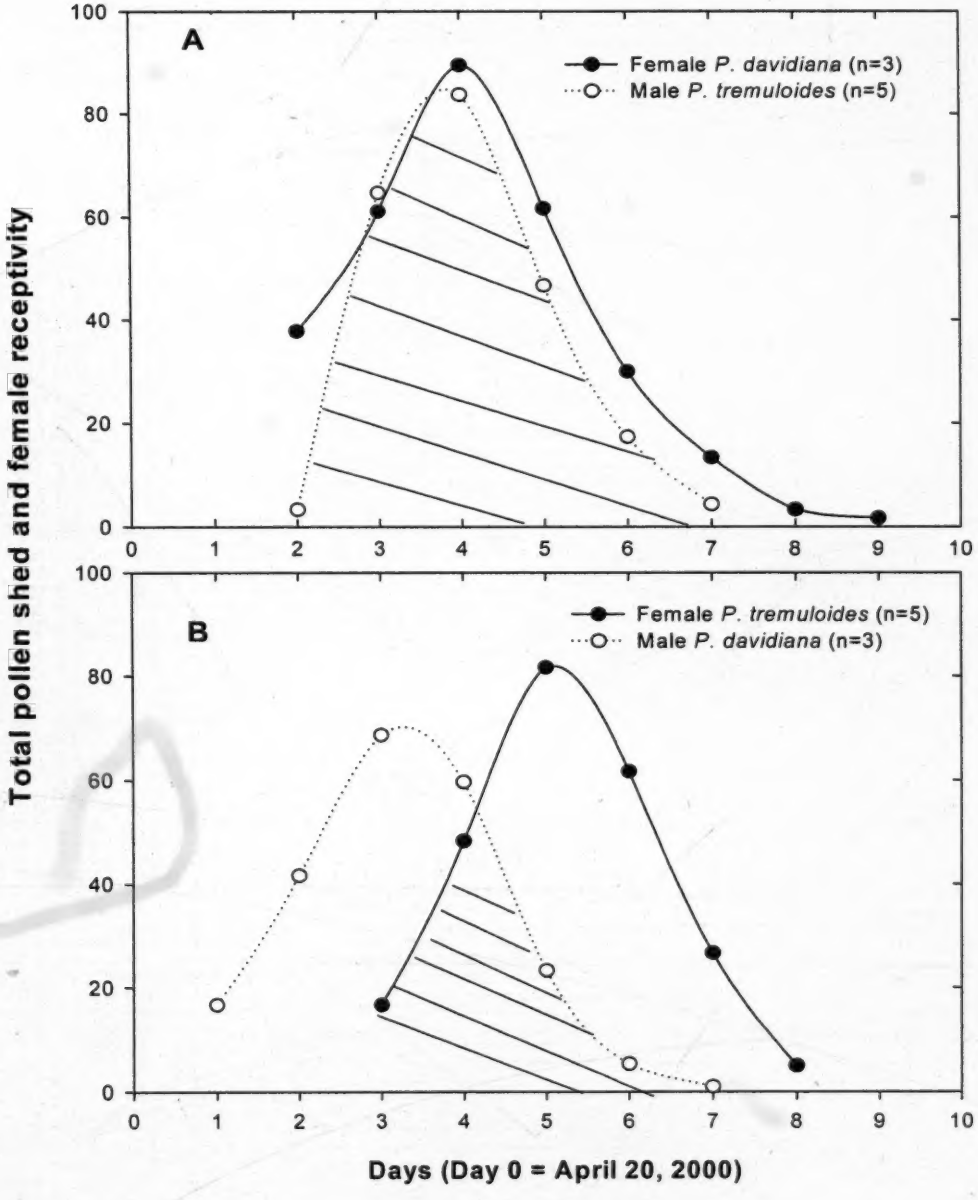
three days (depending on the clone) prior to the peak day of pollen release of *P. davidiana.*

Figure 2-6. Variation (by day) in flowering phenology (during spring 2000) of native and non-native poplars in Athabasca. The horizontal lines show catkin development from bud break to dehiscence and the horizontal bars show active pollen shed and female receptivity. *P. balsamifera* (Pb); Hybrid poplar (Hp); Russian (Rs); Walker (Wk); Brooks6 (Br6); Brooks1 (Br1); Northwest (NW); Manitou (Ma); Tristis (Tr); *P. tremuloides* (Aw); *P. davidiana* (dav). Numerical numbers for *P. balsamifera, P. tremuloides,* and *P. davidiana* represent clone number. Peak day of pollen shed and female receptivity ; Female not fertilized ; Female fertilized Total pollen release: 25-50% ; 50-75% ; 75-100% .



Red line (day 15) = overlapping flowering patterns between the aspens and poplars.
Blue line (day 16) = overlapping flowering patterns between *P. davidiana* (dav 37) and male poplar clones.
Green line (day 24) = Late flowering patterns of both Walker and Manitou.

Figure 2-7. Mean distribution of total pollen shed and female receptivity (stage III) for A) female clones of *P. davidiana* and male clones of *P. tremuloides*, and B) female clones of *P. tremuloides* and male clones of *P. davidiana*. Zones of overlap are denoted by \\ .



Populus (P) tremuloides and *P. davidiana* flowered approximately one week prior to *P. balsamifera* and the hybrid poplars (Figure 2-6 and 2-8). The latest flowering *P. davidiana* clones (dav 38, 42, 43) were still shedding pollen (approximately 15%) on day 15 (April 28), which was the same day as the initial receptivity (approximately 35%) observed in six of the 10 female *P. balsamifera* clones (Pb 5, 8, 9, 10, 14, 16) and one female hybrid clone (Russian) (Figure 2-6 and 2-9A). In addition, the latest flowering female *P. davidiana* clone (dav 37) was receptive (approx. 10%) on day 16 (April 29), at which time there were approximately 13 of the 15 poplar clones (both native and hybrid) actively shedding pollen (from 30% to 60%, depending on the clone) (Figure 2-6 and 2-9B).

Figure 2-8. Mean distribution of total pollen shed and female receptivity for native, non-native and hybrid poplars for A) Athabasca and B) Fort Saskatchewan where *P. balsamifera* (Pb); *P. tremuloides* (Aw); *P. davidiana* (Pd); Hybrid poplar (Hp); female clones (F); male clones (M).

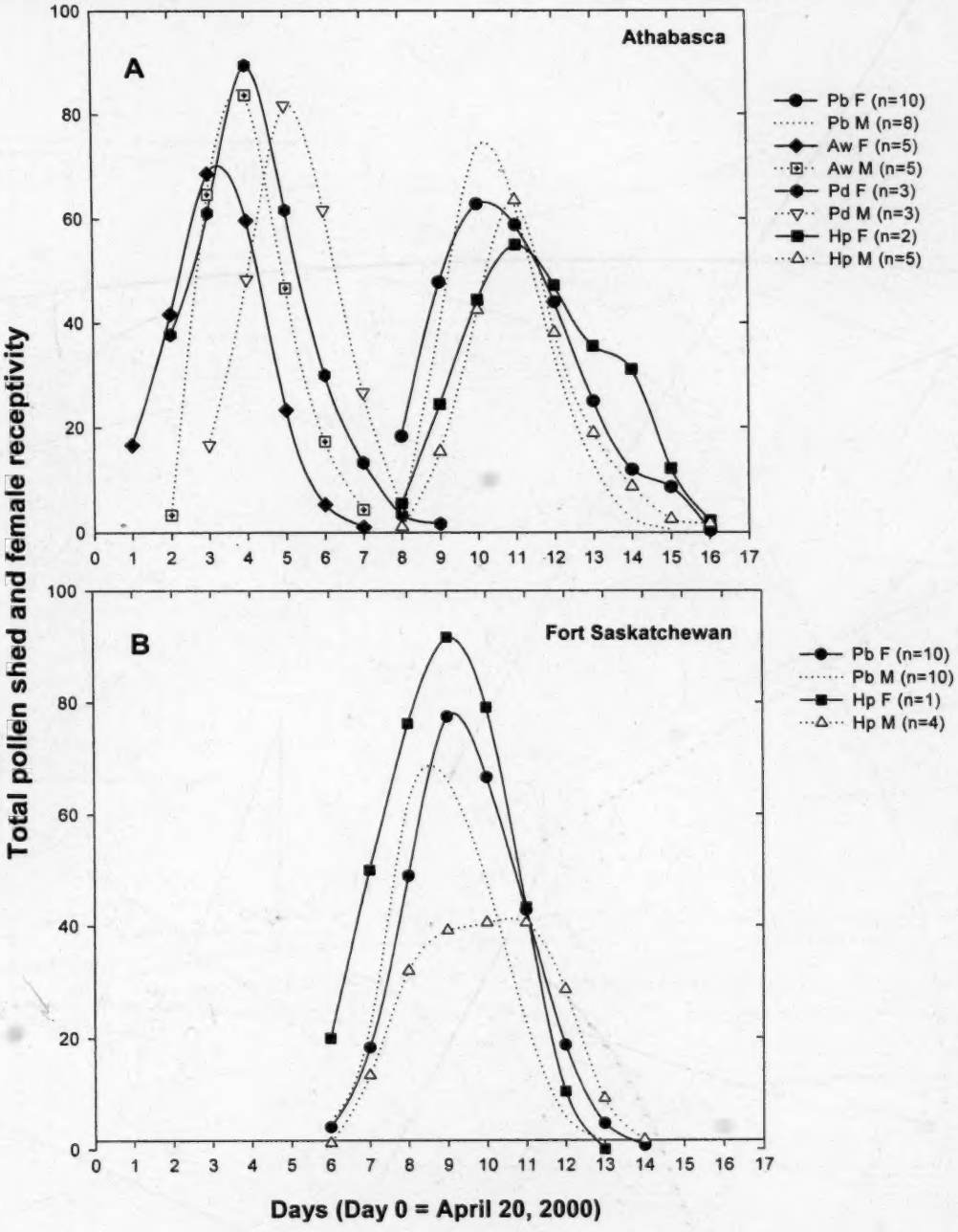
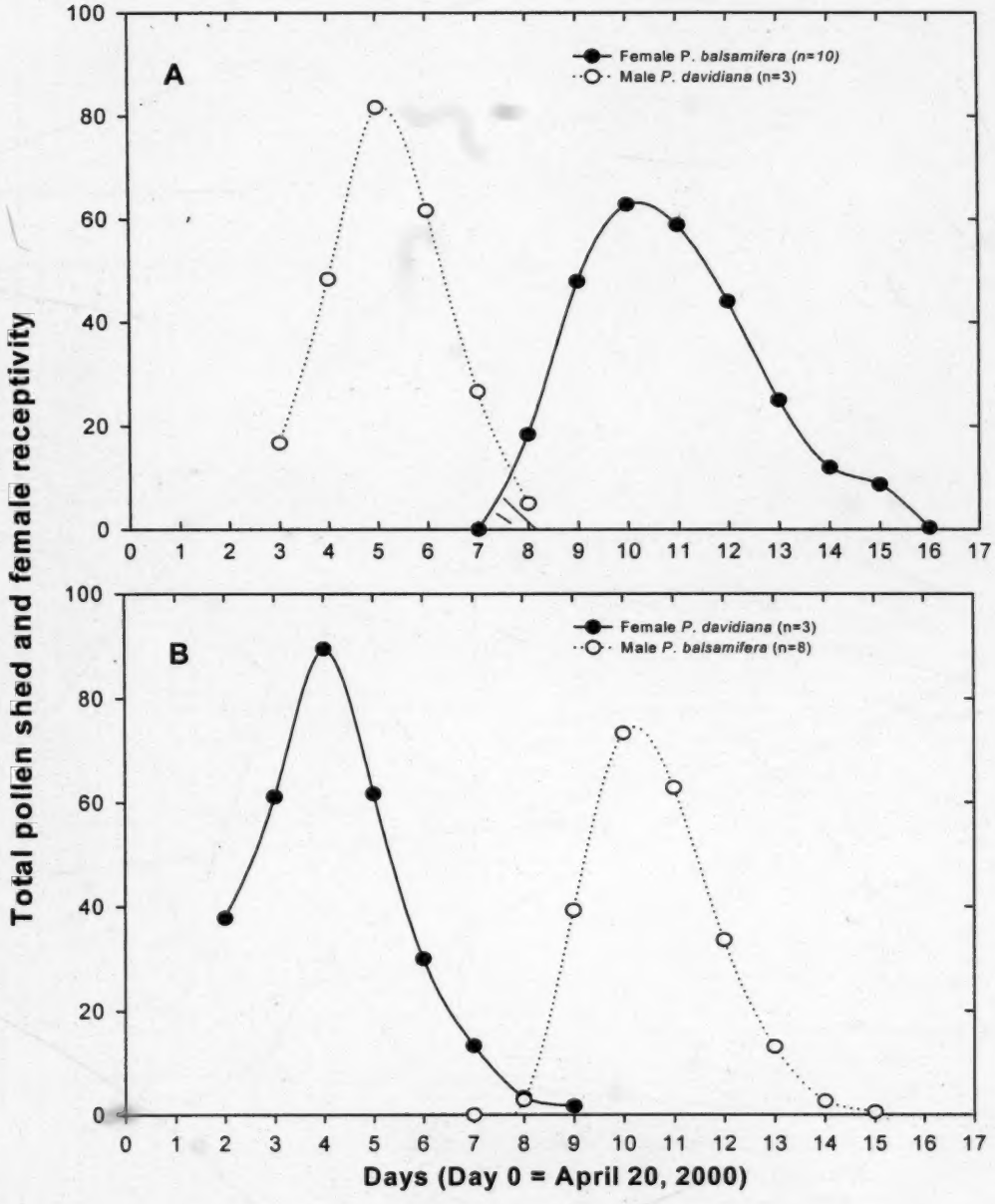


Figure 2-9. Mean distribution of total pollen shed and female receptivity for A) female *P. balsamifera* and male *P. davidiana*, and B) female *P. davidiana* and male *P. balsamifera*. Zones of overlap are denoted by \\\ .



The poplars (P. balsamifera and hybrid poplars) in Ft. Saskatchewan and Athabasca: In general, the flowering of both male and female poplar clones (native and non-native) in Ft. Saskatchewan occurred two days earlier than in Athabasca (Figures 2-8). For the majority of *P. balsamifera* and hybrid poplar clones, the timing of peak pollen release and female receptivity (stage III) occurred over a time span of three days in both Athabasca (Day 16, 17, 18) and Ft. Saskatchewan (Day 15, 16, 17), demonstrating a strong overlap in the flowering patterns (Figure 2-6, 2-10, and 2-11), except for two clones. Walker and Manitou exhibited extended flowering patterns relative to the other native and hybrid poplar clones (Figure 2-6 and 2-12). The mean date of peak fertility for both Walker and Manitou was on Day 21 (May 4th), which was between two to four days later than the majority of *P. balsamifera* and hybrid poplar clones (both sexes), except for one balsam poplar clone. The mean date of peak fertility for *P. balsamifera* clone #17 was one day earlier (day 20) than the date of peak fertility for both Walker and Manitou (Figure 2-6).

The Northwest hybrid (in both study areas) exhibited an atypical flowering pattern where bud break occurred six days earlier than bud break of all other native and hybrid poplar clones (both sexes) (Figure 2-6, and 2-10). However, the initial timing and duration (number of days) of pollen release was similar to the flowering patterns of all other male clones (Figures 2-6 and 2-10).

Figure 2-10. Variation (by day) in flowering phenology (during spring 2000) for native and non-native poplars in Ft. Saskatchewan. The horizontal lines show catkin development from bud break to dehiscence and the horizontal bars show active pollen shed and female receptivity. *P. balsamifera* (Pb); Hybrid poplar (Hp); Russian (Rs); Brooks6 (Br6); Brooks1 (Br1); Northwest (NW); Numerical numbers for *P. balsamifera* represent clone number. Peak day of pollen shed and female receptivity ; Female fertilized ; Total pollen shed 50-75% ; 75-100%.

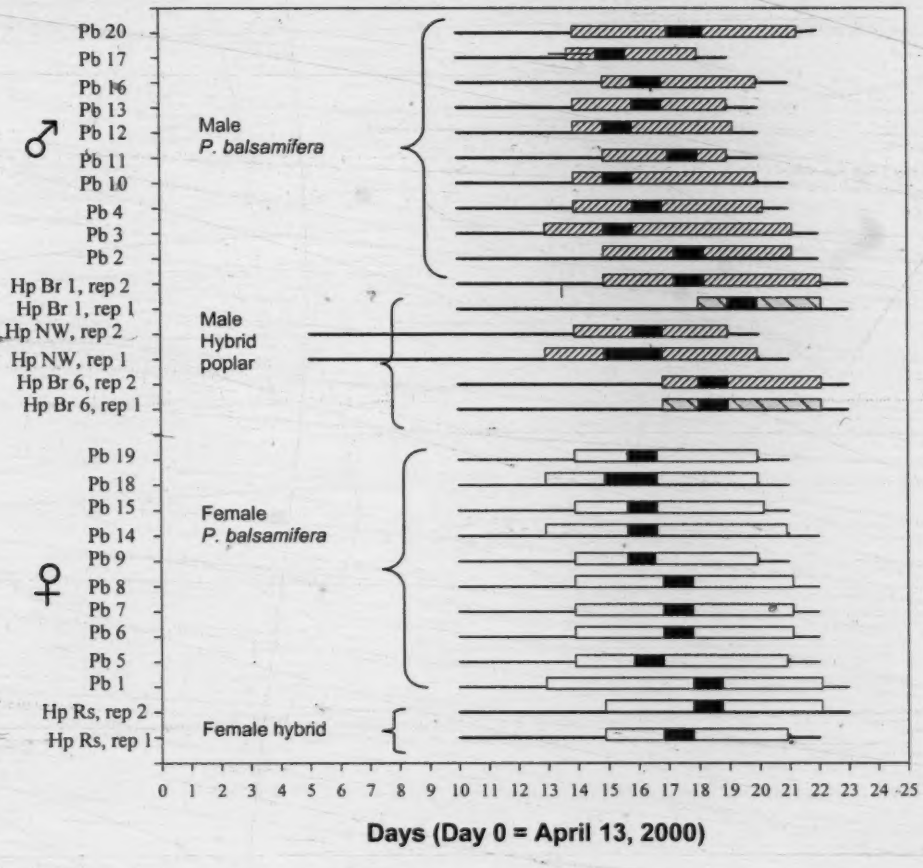


Figure 2-11. Mean distribution of total pollen shed and female receptivity for A) native female *P. balsamifera* and male hybrid clones and B) female hybrid and native male *P. balsamifera* clones in Athabasca, and for C) native female *P. balsamifera* and male hybrid clones and D) female hybrid and native male *P. balsamifera* clones in Fort Saskatchewan. Zones of overlap are denoted by \\\\ .

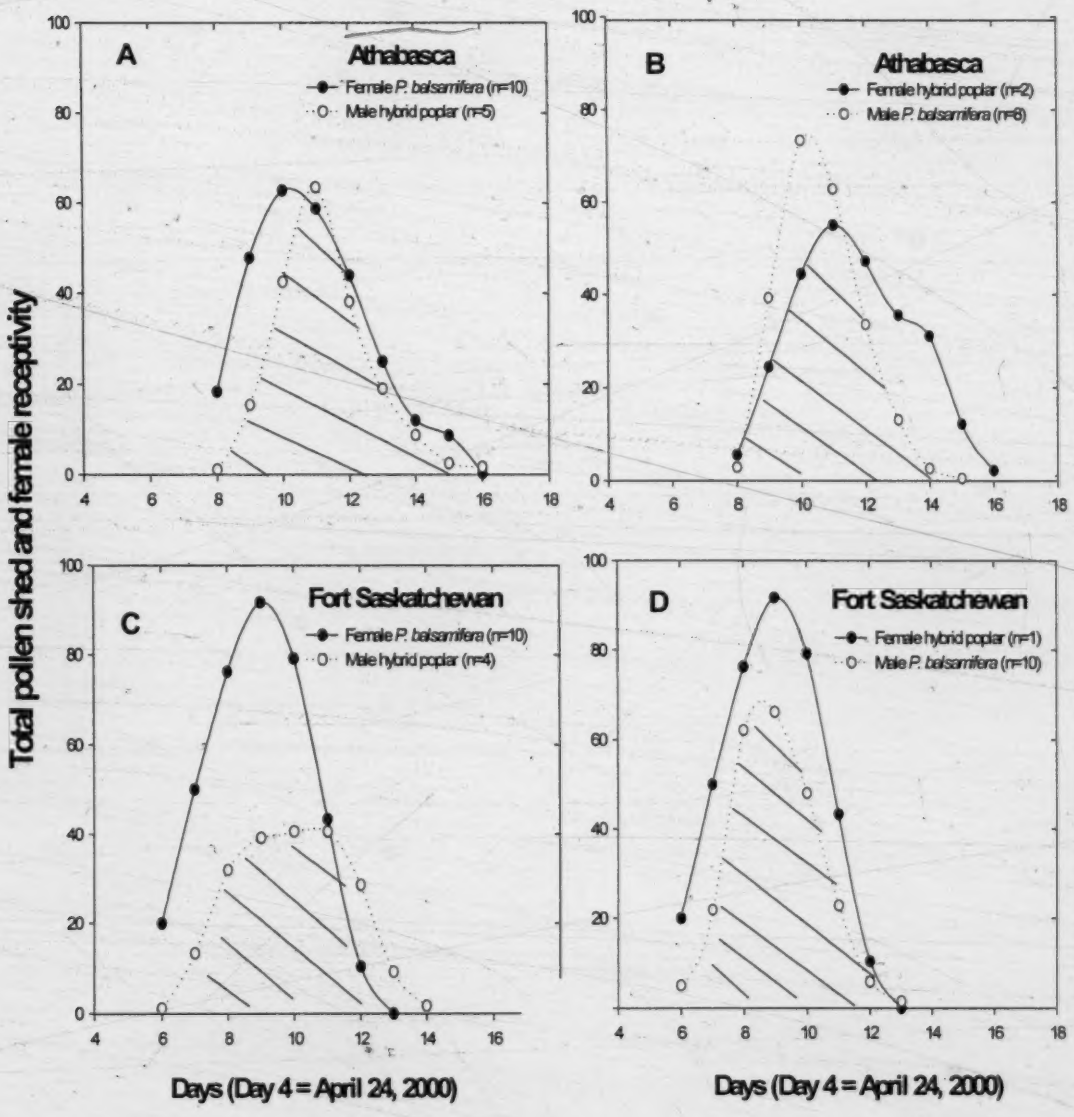
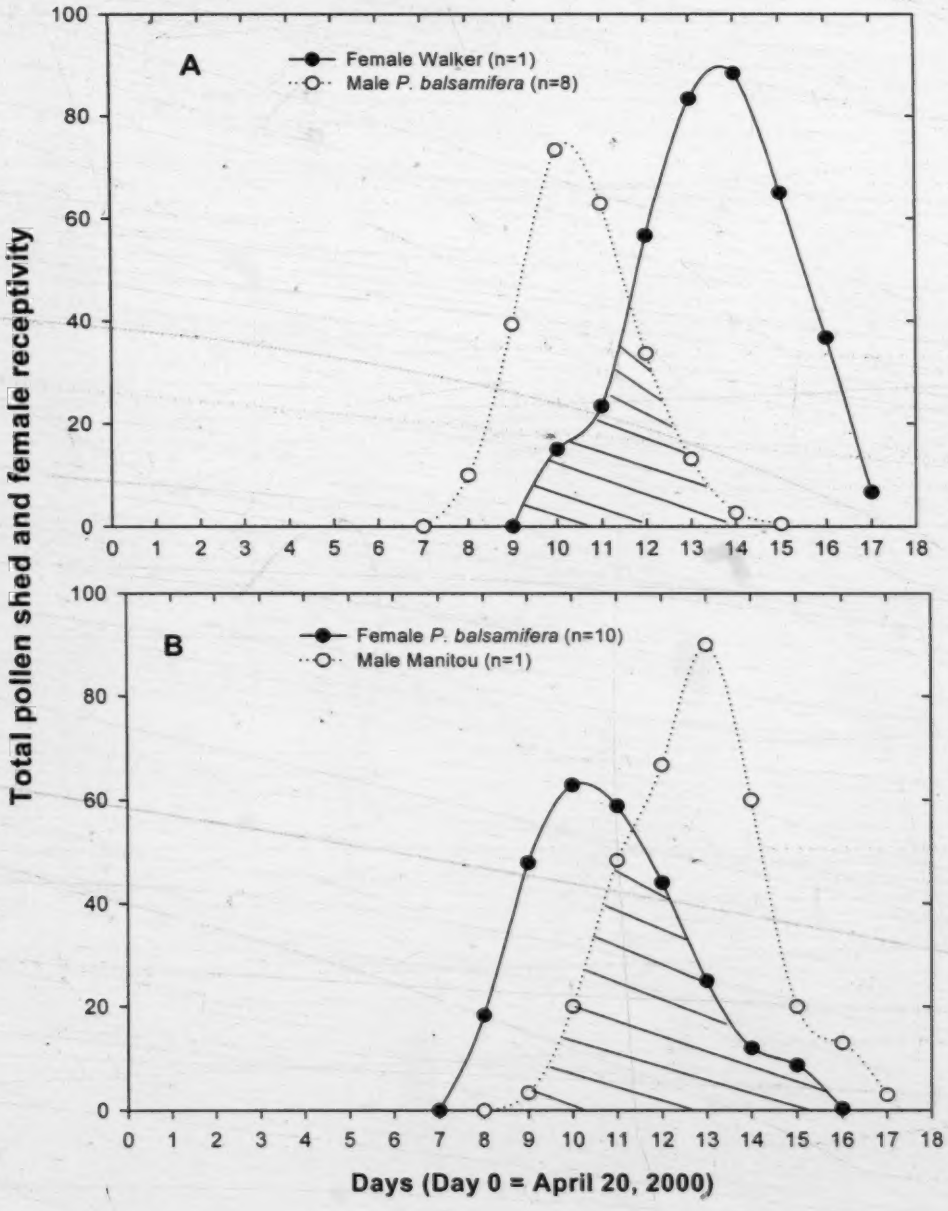


Figure 2-12. Mean distribution of total pollen shed and female receptivity for A) female Walker and native male *P. balsamifera*, and B) native female *P. balsamifera* and male Manitou. Zones of overlap are denoted by \\\ .



2.4. DISCUSSION

2.4.1. *Overlapping flower patterns and the potential for hybridization*

The flowering time between *P. tremuloides* and *P. balsamifera* has been previously described as an effective isolating mechanism, as *P. tremuloides,* on average, flowers one week prior to *P. balsamifera* (Ronald et al. 1973). In this study, observations (one year only) confirm little difference in flowering between the two species (i.e. number of days). Initially, the first signs of pollen release and female receptivity for clones of *P. tremuloides* were seven and eight days prior to the clones of *P. balsamifera*. However, the latest flowering *P. tremuloides* clones and the earliest *P. balsamifera* and hybrid poplar clones were separated by only one day in contrast to the six days reported in other studies (Ronald et al. 1973). This small difference can be explained by the yearly patterns of daily temperatures and accumulation of temperatures above 12^0C, which *P. tremuloides* flowering depends upon (Bassett et al. 1961; Andrejak and Barnes 1969; Einspahr and Winton 1977; Perala 1990), resulting in a potential for overlapping flowering times between *P. tremuloides* and *P. balsamifera*. However, it is well known that these two species are not genetically compatible and that crosses between sections Populus and Tacamahaca are difficult in nature (even under greenhouse conditions / see Figure 1-1) (Zsuffa 1975).

Since *P. tremuloides* and *P. davidiana* are from the same taxonomic section (Populus), it is believed that *P. davidiana* is also not genetically compatible with either *P. balsamifera* or hybrid poplars (used in this study), even though the reproductive phenophases between *P. davidiana* and *P. balsamifera* exhibit a short period of overlap. To illustrate this point, one-female *P. davidiana* clone (Dav 37) was receptive for two days while *P. balsamifera* and hybrid poplar clones were actively shedding pollen. In addition, three male clones of *P. davidiana* were actively shedding pollen for one-day, while seven clones of *P. balsamifera* and one hybrid clone were first receptive.

The aspens (P. tremuloides and P. davidiana): In general, the flowering phenology of both *P. tremuloides* and *P. davidiana* (both sexes) is synchronized. Initial timing and duration of pollen release of *P. tremuloides* corresponds well with the timing and duration of female receptivity of *P. davidiana*, showing an increased likelihood of producing hybrid seed compared to the reverse situation. However, only five female

clones of native *P. tremuloides* were sampled. If more clones were selected for observation, then the likelihood of capturing a wider range of flowering of native clones would probably increase, which in turn would increase the potential for early flowering male *P. davidiana* clones to release pollen and hybridize with late flowering female *P. tremuloides*.

The poplars (P. balsamifera and hybrid poplars): Native and non-native poplar clones in the Ft. Saskatchewan study area were geographically isolated from the clones in Athabasca by 135 km (minimum) and 280 km (maximum), and the flowering patterns of all clones in the Ft. Saskatchewan area were two days earlier than the flowering patterns of both the native and non-native poplar clones in Athabasca. On average, Ft. Saskatchewan was several degrees warmer than Athabasca, which could have contributed to the difference of two days in flowering patterns (Figure 2-8). Regardless, the phenological flowering patterns of both *P. balsamifera* and hybrid poplar clones from each study area were strongly synchronized, demonstrating a potential for hybridization, except for the last few days, when the female hybrid poplar clone Walker (in Athabasca) was still receptive and there was no available native pollen (from the clones observed in this study), except for pollen being actively released from the hybrid Manitou. Since Manitou is the male progeny of Walker, it is not surprising to see a similar flowering pattern being expressed, as flower phenology is believed to be under moderate to strong genetic control (Farmer 1966; Cannell 1989).

In addition to overlapping flowering patterns influencing the potential for hybridization, environmental factors such as rain and wind can influence the probability of hybridization. Rain, cool air temperatures, and an increase in relative humidity can impede the dispersal of pollen more than it hinders the receptivity of females; therefore, fertility gaps can occur between male and female trees, decreasing the chance of fertilization (Sarvis 1962). However, wind enhances the distribution of pollen, as pollen grains can be carried for longer or shorter distances until they land on the ground, in water, or by chance on a receptive female (Sarvis 1962). In Athabasca, there were two female clones (Pb1 and Russian) that developed receptive flowers (stigmas), but fruit did not develop even though both native and hybrid male poplar trees were located within

50m of both females. However, fruit was observed on these same females the following spring.

The potential for hybridization between native and non-native poplars exists, based on similar flowering patterns, if all other environmental conditions and genetic compatibilities are met. Therefore, the potential for hybrid seed getting established exists.

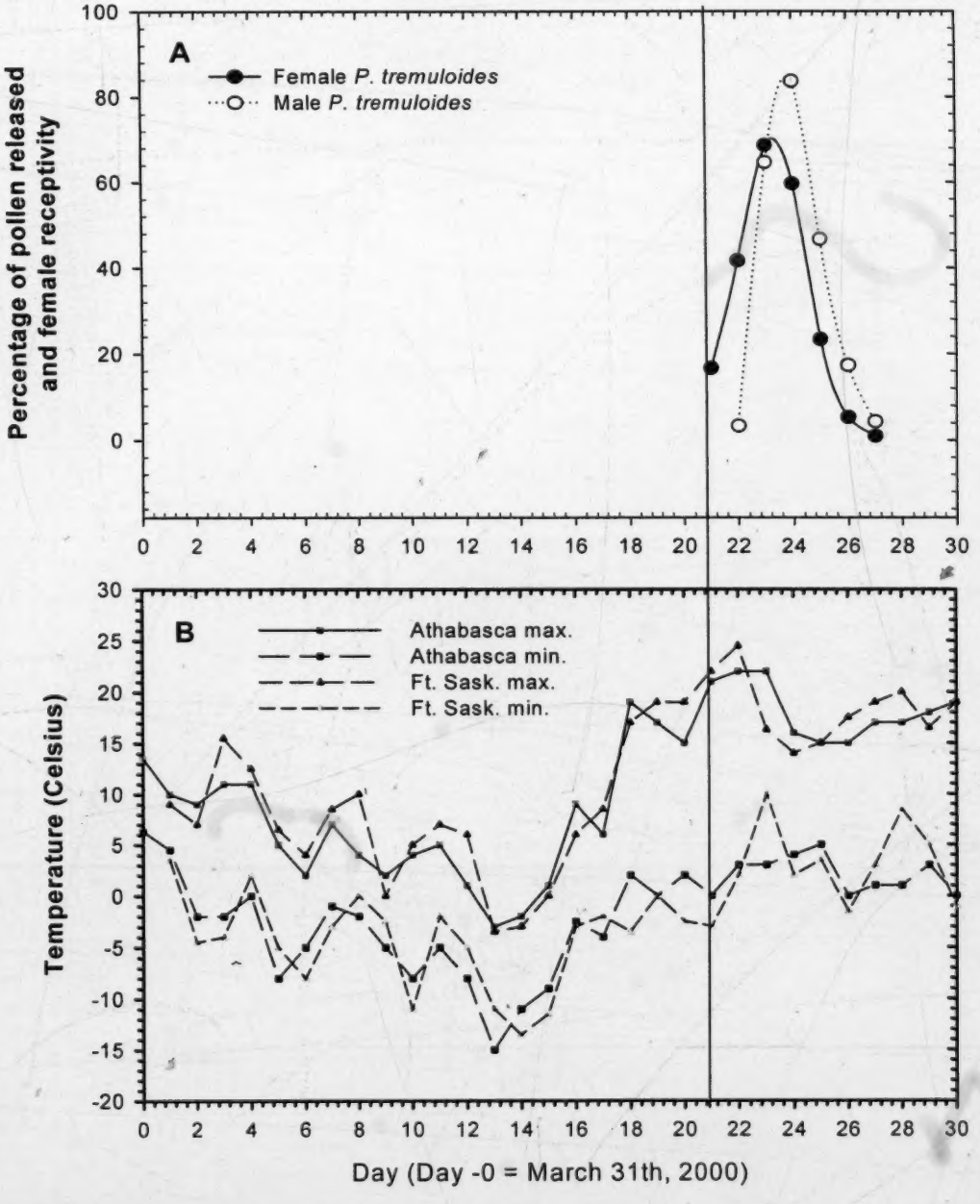
2.4.2. *Flowering phenology*

Cannell (1989) stated that flowering events of forest trees are both highly heritable and adaptive, since a tree's ability to survive and express reproductive fitness depends upon the timing of reproductive and vegetative bud flush, and the length of the growing season. For example, the temperature requirements for bud burst of *Pseudotsuga menziesii* can be unfavorable when trees of a given provenance are planted a few hundred kilometers from their adaptive range (Cannell 1989). Similarly, *P. davidiana* clone #41 (used in this study) and numerous other *P. davidiana* clones (Al-Pac's plantation) seem to be mal-adapted, as they exhibited frozen reproductive buds in 2000. There are many factors contributing to adaptation and it would be difficult to determine exactly why *P. davidiana* was more susceptible to the freezing spring temperatures in north-central Alberta compared with native aspen. *P. davidiana* was selected for this area based on its adaptation to the climatic regimes of northern China, which was supposed to have similar climatic regimes to those of north-central Alberta.

In nature, trees are adapted to local climatic regimes where the onset of spring flower development is directly dependent on both a period of cold dormancy and an accumulation of warming spring temperatures (Lindsey and Newman 1956; Ahlgren 1957; Linkosalo 2000; Beaubien and Freeland 2000; Chuine et al. 2000). For the species *P. tremuloides,* air temperatures of 12^0C or more over a period of six days is believed to stimulate the onset of pollen release and female receptivity (Perala 1990). In this study, *P. tremuloides* released pollen after the sixth and seventh day (April 22 and 23) (depending on the clone) of exposure to air temperatures of 12^0C or warmer (Figure 2-13). In terms of the initial timing (date) of pollen release, a similar study, during the 1950's in northeastern Minnesota, found *P. tremuloides* to release pollen on April 29 (earliest) and May 5 (latest) (Ahlgren 1957). At a glance, the timing of pollen release

between this study (April 22 and 23) and the one in Minnesota (April 29) appears to be wide, but during the 1950's, the maximum air temperature in Athabasca (in April) was around 9.5^{0}C (Environment Canada 1981); therefore *P. tremuloides* probably would have flowered in Ft. Saskatchewan in early May (somewhat later than *P. tremuloides* in Minnesota).

Figure 2-13. A) Initial date recorded for pollen release and female receptivity for *P. tremuloides* (in Athabasca), and B) Minimum and maximum temperatures for Fort Saskatchewan and Athabasca from March 31 (Day 0) to April 30 (Day 30), 2000.



Horizontal line refers to the mean daily temperature of 12^{0}C, and the vertical line indicates the date *P. tremuloides* first releases pollen and had receptive females.

Weather data from Environment Canada for the months of March and April, 2000 (Data requested by phone).

Beaubien and Freeland (2000) claim April 13 was the average date for initial pollen release over a 20-year period (1973-1996 with 4-years missing) for *P. tremuloides* found within a 10,000 km^2 area around Edmonton (located approximately 200 km south of Athabasca, and 30 km southwest of Ft. Saskatchewan). Assuming observations were made within the city limits of Edmonton, where the air temperature is approximately 1.6^0C warmer in downtown Edmonton than 10 km outside the city limits (Beaubien and Freeland 2000), the timing of initial pollen release of *P. tremuloides* located outside of the city would be expected to be approximately two days later than trees located in the city, and then adding another two days for the late flowering patterns observed in Athabasca compared to Ft. Saskatchewan, April 17 would be the projected date of initial pollen release according to Beaubien and Freeland's (2000) data for *P. tremuloides* in Athabasca. In this study, the first sign of pollen release for *P. tremuloides* clones was on April 22, a phenological gap (timing of pollen release) of approximately five days between this study and Beaubien and Freeland's (2000). This phenological gap is relatively small considering that the two studies were conducted in different years; Beaubien and Freeland's (2000) study was conducted over a 20-year period, whereas this study was based on one field season (2000).

2.4.3. *Summary and conclusion*

Flowering data was collected for 40 clones of native balsam, 10 clones of native aspen, six-clones of *P. davidiana*, and seven-hybrid poplar clones. These data proved useful in identifying flowering patterns for individual clones and the zone of overlap between the flower patterns of native and non-native poplar clones. The flowering patterns between male and female clones within the species *P. tremuloides, P. davidiana, P. balsamifera* and within the hybrid poplars varied and overlapped, suggesting a potential for hybridization between *P. tremuloides* and *P. davidiana,* and between *P. balsamifera* and hybrid poplars. Although, the late flowering patterns of the hybrid poplars Walker and Manitou do not directly overlap with the flowering patterns of the native balsam poplar clones observed in this study. Therefore, these late flowering clones, are likely to hybridize with native balsam poplar, but perhaps to a lesser extent.

To conclude, the potential for hybridization between native and non-native poplars exists only because of overlapping flowering times, and then only if all other environmental conditions and genetic compatibilities are met. The next step is to determine if native and non-native poplars (used in this study) are genetically compatible.

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CHAPTER THREE: Greenhouse Breeding Experiment

3.1. INTRODUCTION

3.1.1. *Poplar breeding*

Poplars are a group of species that have been shown to readily produce viable seed. However, there is considerable complexity in the degree of compatibility. To determine whether hybridization is possible between native and non-native poplars in Alberta it is important to get a general understanding of the complexity of breeding two species from different taxonomic sections, and breeding hybrids with either hybrids or pure species. In poplar breeding programs, the fertility of crosses between two poplar species can vary, and the direction of the cross can be fundamentally important (Zsuffa et al. 1999). For example, it is well known that the cross of *Populus (P.) deltoides* as the female parent and *P. nigra* as the male parent is fertile, whereas the reciprocal cross typically produces non-viable seed (Stettler et al. 1996). Similarly, the crossing of *P. deltoides* x *P. balsamifera* (female parent is always listed first), *P. deltoides* x *P. maximowiczii*, and several crosses using *P. maximowiczii* as the female parent with *P. balsamifera, P. nigra,* and *P. x jackii* as male parents can produce viable seed, while the reciprocal crosses fail (Zsuffa et al. 1999). These one-way compatibilities are believed to be associated with post-zygotic barriers, resulting in the development of empty seed and premature capsule abscission (Stettler et al. 1996; Zsuffa et al. 1999).

Other types of incompatibility barriers exist, such as pre-fertilization barriers and hybrid inviability. Pre-fertilization barriers also cause premature capsule abscission, whereas hybrid inviability refers to weak seedlings, expressing poor development and lack of vigor during the first few months or years (Stettler et al. 1996). The genetic mechanisms underlying the three incompatibility barriers are not clearly understood, but the belief is that the processes responsible for the incompatibility barriers get more complex as the process moves from pre-fertilization to post-zygotic barriers to hybrid inviability (Stettler et al. 1996; Zsuffa et al. 1999). In general, successful crossability studies are published far more often than unsuccessful studies, as breeders tend to blame their failed results on poor technique, inadequate material and/or bad environmental

conditions rather than on possible genetic incompatibility barriers (Stettler et al. 1996), as breeding poplars requires stringent procedures (Stanton and Villar 1996).

3.1.2. *Objective*

There are no published reports on crossing native *P. balsamifera* (Tacamahaca) with the inter-specific hybrids (Aigeiros and Tacamahaca) described in Chapter two. Therefore, the objective of this breeding experiment was to determine if native balsam poplar and locally grown non-native poplars are genetically compatible under greenhouse conditions by determining if viable hybrid seed could be produced. Four mating combinations were used (native x native, native x hybrid, hybrid x native, and hybrid x hybrid), using cut branch material from both *P. balsamifera* and hybrid poplar clones (male and female), which were forced to flower in the greenhouse. Pollen was then collected and applied to receptive females.

Pollen from four *P. balsamifera* clones was collected, and mixed together to produce one poly-mix (source) of pollen for breeding with the female native and hybrid poplar clones. This was done for two reasons: 1) to increase the likelihood of successful crosses, because to rely on a single source (from one clone) of native pollen may decrease the breeding success due to natural inter-clonal variation, and 2) we were interested in cross breeding the species, not individual clones within the species.

The species *P. tremuloides* and *P. davidiana* were not used in this experiment as the plantation of *P. davidiana* located at Alberta-Pacific Forest Industries Inc. (Al-Pac) was only six-years-old and flowering for the first time (too few buds), and since *P. tremuloides* and *P. davidiana* are from the same taxonomic section they can hybridize, but they are not fertile with species from other taxonomic sections (Figure 1-1).

To attain the goal of this study, two experienced poplar breeders (Serge Morin from the Quebec Ministry of National Resources (QMNR), and Dan Walker from the Prairie Farm Rehabilitation Administration (PFRA) in Indian Head, Saskatchewan) were consulted, and the procedures outlined in Stanton and Villar (1996) were used to understand the protocol and stringent techniques needed to ensure viable hybrid seed was produced, if parent trees were compatible. Stanton and Villar (1996) outlined the basic 'pot in pot' technique and a variation of it for breeding poplars from sections Aigeiros and Tacamahaca. Both Serge Morin and Dan Walker used the 'pot in pot' method, but

modified it as they found other procedures improved their breeding success rate. The technique outlined in the Material and Methods (see section 3.2) uses a combination of all three sources with additional modifications.

According to Serge Morin, a breeding success rate of 50% (at best) can be expected if all environmental (artificial) conditions and biological compatibilities are met to produce viable seed. Hybrid seed was collected and tested for its viability by determining the percentage of normal and abnormal seedlings, and the percentage of dead seed.

3.2. MATERIAL AND METHODS

The "cut branch" technique (Einspahr 1960) was used to force flowering in male clones (Zsuffa 1975; Stanton and Villar 1996). This technique is also used for most female *Populus* species, as seed develops quickly (2-3 weeks) (Zsuffa 1975; Stanton and Villar 1996). However, Aigeiros and Tacamahaca poplars release mature seed between six to eight weeks after pollination (Zsuffa 1975; Stanton and Villar 1996) and therefore, these species require a functioning root system to meet the demand for moisture and nutrients required for the longer seed development phase (Schier and Campbell 1976; Cunningham and Farmer 1984).

3.2.1. *Branch collection*

On January 15, 2001 approximately five dormant branches (between 1.5 – 2.0m long) with reproductive flower buds were collected from eight *P. balsamifera* (4 males and 4 females) and seven hybrid poplar (5 males and 2 females) clones using a 5.5m pruning pole and a 2m-ladder (Table 3-1). Lateral branches (approximately 1m in length) were cut from the main (cut) branch, tied together at the base, and labeled (clone name and location). The base of each bundle of branches was wrapped with Mountain Mist Gold-Fuse Batting (114 x 152cm) (Stearns Technical Textiles Company. Cincinnati, OH) soaked with distilled water to prevent desiccation, covered with a small white kitchen garbage bag, and sealed with duct tape. To protect the reproductive flower buds from damage in transit, a large clear plastic drum liner (56cm + 46cm x 152cm x 4ml)

(Innovative Plastics, Edmonton, Alberta) was placed over each bundle of branches, and branches were stored at -8^0C.

To ensure all pollen was collected prior to female receptivity, male branches were prepared first, followed by female branches one week later. Female branches were placed in a freezer at -27^0C for one week at the Northern Forestry Centre in Edmonton, Alberta, while male branches were placed in a growth chamber at the University of Alberta to commence flower forcing.

Table 3-1. Breeding material, sex, and location of both male and female poplar clones used in the breeding experiment.

Breeding material[1]	Sex[2]	Location
4 native balsam poplar	F	Ft. Saskatchewan
Russian	F	Ft. Saskatchewan
Walker	F	Al-Pac's[3] mill site
4 native balsam poplar	M	Ft. Saskatchewan
Brooks #1	M	Ft. Saskatchewan
Brooks #6	M	Ft. Saskatchewan
Northwest	M	Ft. Saskatchewan
Tristis	M	Athabasca
Manitou	M	Al-Pac's mill site

1. Parentage of hybrids see Chapter II, Material and Methods
2. F = female, M = male
3. Al-Pac = Alberta-Pacific Forest Industries Inc.

3.2.2. *Growth chamber preparation, and male flower forcing*

On January 16, 2001 a growth chamber was set-up with a 14h photoperiod with low light intensity (5 incandescent lights @ 24 W each, and 12 fluorescent lights at 40 W each), relative humidity (RH) of 80%, and a diurnal temperature of 10^0C (day) and 8^0C (night). The diurnal temperature was increased daily by 2^0C to a maximum of 20^0C to mimic natural temperatures and promote pollen development. Other requirements for normal flower maturation as well as viable pollen were to avoid temperatures above 26^0C, avoid direct sunlight and maintain low humidity. Although high humidity is necessary during anthesis, low humidity is preferred during pollen release.

On January 17, 1cm was cut off the basal end and branches were placed in 20 litre pails of cold water with one clone per bucket. Approximately 12-branches per clone were selected based on branch shape, number of reproductive buds, and position of buds. Branch ends were loosely tied to help keep them together, and both the plastic buckets and branches were labeled (clone name and location). A clean, clear plastic drum liner was placed over the branches, leaving a large opening at the base of the bucket. The plastic liner was used to capture pollen that fell during the process of pollen collection and provided a barrier to contamination. An air bubbling system was installed in each bucket using small air pumice stones, and air tubing with approximately 120-PSI of forced air. Aerating the water helped to maintain water quality, which in turn helped to prevent the plugging of vascular tissue. On a weekly basis, all branch ends were re-cut one centimeter from the base.

To make room for female branches in the growth chamber, male branches were moved into a regular greenhouse on January 25th. The greenhouse temperature was maintained at 20^{0}C (day) and 10^{0}C (night), with an average RH of 70%. Direct sunlight was filtered by attaching white shade cloth to the inside windows.

3.2.3. *Pollen collection*

To prevent pollen contamination, the technician's body and work area were misted with water (using a small water bottle) between pollen lots to burst pollen grains. A facial mask was worn during the collection of pollen as microscopic pollen grains are considered an allergen. Disposable plastic hand gloves were also worn and a new pair was worn for the handling of each pollen lot.

When catkins were fully elongated (approximately 5cm) and anthers started to change color from bright red to a powdery yellow (pollen release) buckets were removed from the greenhouse and placed in a room (21^{0}C, 30% RH) to finish ripening. Pollen was collected by cutting a large opening at the top of the drum liner and removing ripe catkins using a small hand pruner, carefully cutting catkins into a catch basin. Inevitably, catkins and pollen grains fell to the bottom of the liner, and were collected by lightly tapping the sides of the liner and pulling on the liner at the bottom to make a funnel. A small hole was cut in the liner at the tip of the funnel, allowing pollen grains and catkins to fall into the catch basin. This hole was re-sealed by rolling the plastic and attaching a clothes-pin.

Catkins were processed (see 3.2.4. *Pollen extraction and viability test*) and discarded. The plastic drum liner was wrapped around the branches and held together by wooden clothes-pins until the following day. Pollen collection usually took place over two or three days.

3.2.4. *Pollen extraction and viability test*

The development and release of pollen varies within and between catkins (Pregitzer and Barnes 1980; Kaul 1995). Therefore, catkins expressing both red (anthers) and yellow (pollen release) colors were collected and processed by placing catkins in a labeled brown paper bag for 24-hours for further ripening. In each paper bag, catkins were placed in a single layer to ensure air circulation between catkins, and placed in a well-ventilated area (over a metal grate) at room temperature. After 24-hours, pollen was extracted again.

A 20-mesh screen (0.84 mm opening from Canadian Standard Sieve Series by W.S. Tyler Co. of Canada Ltd., St. Catherines, Ont.) and a catch basin (attached to the bottom sieve) were used to remove bud scales, floral bracts and catkins from pollen grains. Rubbing catkins together and lightly tapping the container allowed pollen grains to fall into the catch basin, while bud scales and floral bracts remained in the top sieve. Pollen grains were transferred onto a sheet of paper previously folded in the middle to act as a funnel, and assessed as either dry or wet by observing how the pollen flowed. If pollen flowed easily like flour, then it was assessed as dry, but if pollen grains were lumpy and did not flow easily, then it was assessed as wet. Dry pollen grains were poured into glass vials (labeled), sealed with lids, taped (to prevent moisture accumulation), and placed in cold storage at 4^0C in a desicator. Wet pollen grains were dried by pouring pollen lots into glass vials and inserting a piece of cotton at the top of the vial (to act as a breathable lid) and placed over Drierite (a desicant) in a desicator for two or three days at 4^0C. Vials containing wet pollen were monitored daily for ease of flow, and after two or three days cotton was removed and vials were sealed with lids and tape. All pollen lots were then tested for viability.

A 6% sucrose and 5% agar solution was made and poured into sterilized petri-plates, then cooled to room temperature (Mallett and Huybregts 1999). A small amount of pollen from each pollen lot was collected using the end of a fine-pointed tool, and

blown onto the agar mixture (one pollen source per petri-dish) using a photo lens bulb cleaner. Lids were placed on petri-plates and left at room temperature for 48 hrs. Pollen lots were assessed for viability after 48 hours based on the presence of pollen tubes under a high-powered binocular dissecting microscope (WILD - 50-75X) (Mallett and Huybregts 1999).

3.2.5. ***Female flower forcing and preparation of pots***

On January 25, female dormant branches were removed from the freezer and placed outside (-2^{0}C day, -11^{0}C night) in the shade to thaw for 24-hours followed by 24-hours in a growth chamber at 8^{0}C. On January 28, the basal ends of all female branches were cut 1cm and inserted into prepared round plastic pots. Pots were placed in the growth chamber for approximately three weeks where the soil temperature was kept warm (22^{0}C, using heating pads) to stimulate root growth, and the air temperature kept cool (8^{0}C) to prevent catkins from developing. On January 29, all branches were sprayed with dormant oil (20ml/l, by Plant Products, Brampton Ont.) to kill any larvae, which might later emerge and damage the seed during development.

Drainage holes (1cm wide) were melted along the sides (at the bottom) of 10-plastic pots (10-litre), using an electric heating probe. Tomato towers (84cm) were stretched and placed over the outside of the pot by cutting the first two rings of each tomato tower with pliers, and then attached by bending the metal pins at the base into the holes at the bottom of the pots. Each pot was filled with soil media (1/3 perlite, 1/3 peat moss, 1/3 potting soil), which was loosely packed, and pre-treated with a fungicide (Wilson's Soil Insect Dust). One branch per clone was selected (minimum of 10 reproductive buds/branch), labeled, and placed into each pot (each pot labeled with one-pollen source) (Table 3-2). To accommodate the remaining crosses, breeding bags (15cm x 30cm) were used on lateral branches containing sufficient number of floral buds.

Table 3-2. Male and female poplar clones used in the breeding experiment, and all possible mating combinations are denoted by 'x' (replicated twice).

Female / Male	Russian	Walker	Pb 1	Pb 2	Pb 3	Pb 4
Brooks 1[1]	x	x	x	x	x	x
Brooks 6	x	x	x	x	x	x
Northwest	x	x	x	x	x	x
Manitou	x	x	x	x	x	x
Tristis	x	x	x	x	x	x
Pb[2] poly-mix[3]	x	x	x	x	x	x

1. See Chapter II, material and methods for parentage of hybrid clones.
2. Pb = balsam poplar.
3. Pollen collected from 4-native balsam poplar clones were mixed together to form a poly-mix

Sheets of Styrofoam (2.54cm thick) were used to build insulating boxes for the plastic pots (two pots per box). King size heating pads (Sunbeam Corporation (Canada) Ltd. Mississauga, Ont.) were wrapped around each pot and placed inside the Styrofoam boxes to keep soil temperature around 22^0C (Figure 3-1).

Figure 3-1. Female branches labeled with pink ribbon and each pot labeled with one pollen source (blue ribbon). Soil temperature was maintained at 22^0C and air temperature at 8^0C.

3.2.6. *Growth chamber, greenhouse, and breeding chamber preparation for female pots*

Growth chamber requirements for female branches were the same as for the male branches, except diurnal temperatures remained constant (10^0C day, 8^0C night) for approximately three weeks to promote root development prior to flowering. Catkins started to break bud and elongate at approximately day 15 (February 12). As a result, air temperatures were increased daily by 2^0C to a maximum of 20^0C. On February 22, heating pads were removed, and pots were prepared for breeding (see section 3.2.7. *Pollen application*).

To prevent air temperature in the greenhouse from exceeding 26^0C (imperative for the development of viable seed) summer cloud liquid shading (Plant Products Brampton, Ont.) was applied to the outside windows, the roller shade on the roof was lowered, and the ceiling fan turned on. Two thermometers were placed on benches to monitor maximum and minimum air temperatures, and relative humidity.

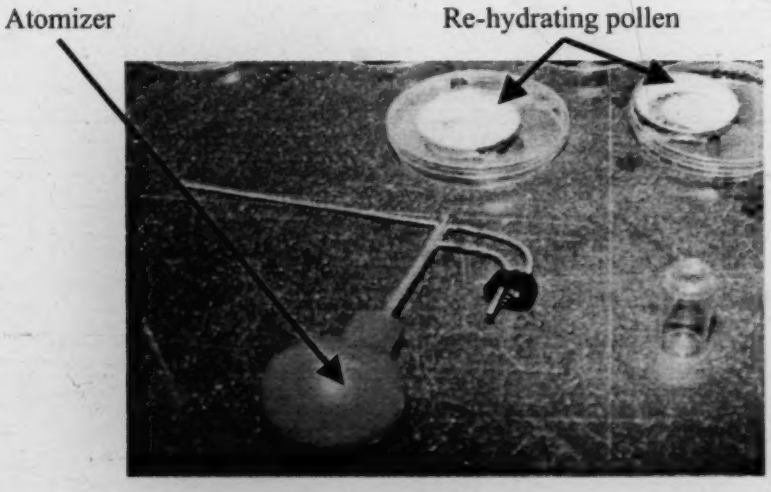
Vegetative buds were reduced to a minimum by removing terminal leader and lateral buds, leaving one or two vegetative buds above the floral catkins. Two or three wooden bamboo stakes (approximately 1m long) were placed inside each pot to support a clean drum liner, making a temporary breeding tent to isolate female flowers and prevent unintentional open pollination. To gain access to catkins, a large slit was made by cutting the liner from the top to the bottom. Entry into the tent was kept closed by rolling the cut edges together and securing it with wooden clothes-pins. To help keep the air temperature inside the tent from rising, three small rectangles (30cm x 15cm) were cut at the top and sides of each tent, and replaced with a double layer of paper towel held by duct tape.

3.2.7. *Pollen application*

On February 22, prior to the application of pollen, all sources of pollen were re-hydrated for approximately one to two hours. A small amount of pollen was removed from each vial and placed onto a small plastic cap, which was then placed on a sponge inside a closed petri-plate filled with a small amount of water (Figure 3-2). Pollen atomizers (or bulb syringe) (supplied and made by Tim Gylander from Weyerhaeuser Company Limited, Drayton Valley, Alberta) were used to blow pollen onto receptive

stigmas (Figure 3-2). During the application of pollen the ceiling fan was turned off, and after each application of a pollen lot, the greenhouse, technician's body and work area (pollen re-hydrating station) were misted with water to prevent pollen contamination. Vials and atomizers were sterilized using 95% Ethanol and air-dried. Four atomizers were used, allowing time for the atomizers to dry between applications of pollen lots. Pollen was applied to receptive stigmas every day for four days.

Figure 3-2. Prior to the application of pollen onto receptive stigmas, pollen was re-hydrated and then applied using an atomizer.



3.2.8. *Seed collection, extraction, and germination test*

Between March 29 and April 9 seed capsules were collected as soon as they initiated dehiscence, then capsules were placed in labeled brown paper bags and air dried at room temperature for three to four days. For ease of handling and longevity, seed was extracted from the white cotton fibers.

Cotton fibers were removed from seed by forcing air into a series of standard sieves, with the arrangement of 20, 40, and 60 mesh screens (0.84, 0.42 and 0.25mm openings, respectively), which created a tumbling motion. The top sieve (0.84mm), containing seed and fluff, was covered with clear plastic and taped to secure the edges. A small hole was made at the top of the plastic to accommodate the 6.0mm diameter air

hose. The fluff remained in the top sieve while seed fell into the middle (0.42mm), and bottom (0.25mm) sieves. Seeds were placed in small glass vials (labeled), sealed with lids and tape, placed in a zip-lock bag, then stored in a non-frost-free freezer at -23^0C. A non-frost-free freezer was used because they remain at a constant temperature.

Seed germination tests were done following the material and methods described by Edwards (1987), which recommends using 100 seeds per replicate and four replicates where possible. However, in this study, there were less than 250 seeds per cross. Therefore, for the crosses producing 150 seeds or more, a germination test consisting of 50 seeds and three replicates was used, and for other crosses with a seed crop of less than 150, the maximum number of seeds produced was used (Table 3-3). A maximum of 50 seeds were carefully placed onto each petri-plate using a pair of tweezers to ensure seeds did not touch each other. To prevent moisture loss, petri-plates and lids were sealed with masking tape. Seedlings were evaluated after three-days by categorizing them as normal, abnormal or dead (Edwards 1987).

Normal seedlings were gently removed from the petri-plates with tweezers and placed on soil media in Hilton root trainer trays (Type 170-4, Spencer-Lemaire, Edmonton, Alberta). Three seedlings were placed in each cavity and placed in a greenhouse under a 16-hour photoperiod and a temperature of 24^0C to encourage root development and growth. These seedlings were then used for leaf measurements (Chapter IV) and DNA extraction to confirm parentage (not part of this thesis).

Table 3-3. Mating combination, breeding cross, and number of replicates used in the seed germination test.

Mating combination	Breeding Cross Female x male	Number of Replicates used	Total number of seeds
Native x Native[1]	Balsam 2 x Balsam[2]	3	150
	Balsam 3 x Balsam	3	150
Native x Hybrid	Balsam 2 x Northwest[3]	3	150
	Balsam 2 x Tristis	3	125
	Balsam 2 x Brooks 1	3	150
	Balsam 2 x Brooks 6	3	150
	Balsam 2 x Manitou	1	55
	Balsam 4 x Manitou	3	150

Hybrid x Native	Russian x Balsam	3	150
Hybrid x Hybrid	Russian x Brooks 1	2	100
	Russian x Brooks 6	1	40
	Russian x Manitou	1	24

1. See Chapter II, Material and Methods for parentage of hybrid clones.
2. Balsam = *P. balsamifera* poly mix (pollen).
3. Pure sp. = pure species of *P. balsamifera*.

3.2.9. *Pollen and seedling assessment*

In the pollen viability test, the observation of at least one pollen tube per pollen lot under a high-powered dissecting microscope was considered sufficient to claim viable pollen was used for the breeding experiment. From the seed germination tests, a mean average for the total number of normal and abnormal seedlings, and dead seed for each replicate within a cross was calculated. Normal seedlings were identified as seedlings exhibiting essential structures (cotyledons and primary root) required to produce normal trees under field conditions (Edwards 1987). Abnormal seedlings were previously described by Edwards (1987) as being defective in one or more ways: 1) damaged seedlings were seedlings without cotyledons and primary root; 2) deformed seedlings had weak, twisted or stunted plumules (rudimentary stem) or development stopped after initial emergence; 3) decayed seedlings showed disease on essential structures.

3.3. RESULTS

3.3.1. *Visual observation*

Populus balsamifera (Pb#1) did not display normal flower development as flowers did not move from stage II to stage III. They appeared pale yellow in color and withered, and during the application of pollen, flowers prematurely fell off the branches. Therefore, there were 30 complete crosses instead of the original design of 36 crosses.

Approximately 20-days from the last pollination date, 18 out of 30 crosses did not produce seed due to insect larvae and premature capsule abscission (Table 3-4). Insect larvae were found burrowing through the green seed capsules in several branches (from different pots) of two *P. balsamifera* clones (#3 and #4). At first these branches appeared

ready to release seed as white cotton fibers were emerging from the green capsules, which was surprising as normal seed development generally takes between 30 and 50 days. Premature capsule abscission occurred shortly after vegetative buds flushed out, and leaf expansion ceased in several branches (from different pots) of two *P. balsamifera* clones (Pb#3 and Pb#4), and two hybrid poplar clones (Russian and Walker).

Table 3-4. Male and female clones used in the breeding experiment, pollen viability results for 6 pollen sources, and the success or failure of each breeding cross denoted by one of the following: Seed collected (X); Females not receptive; Premature capsule abscission; Presence of insect larvae.

M\F Parent[a]	*Pollen Viability*	*Russian*[b]	*Walker*	*Pb #1*[c]	*Pb#2*	*Pb #3*	*Pb #4*
Pb poly-mix[d]	Yes	X	premature capsule abscission	Females not receptive	X	Insect larvae	Insect larvae
Brooks 1	Yes	X	premature capsule abscission	Females not receptive	X	Insect larvae	premature capsule abscission
Brooks 6	No	premature	premature capsule abscission	Females not receptive	X	premature capsule abscission	premature capsule abscission
Northwest	Yes	capsule	premature capsule abscission	Females not receptive	X	Insect larvae	Insect larvae
Tristis	Yes	X	premature capsule abscission	Females not receptive	X	premature capsule abscission	X
Manitou	Yes	X	premature capsule abscission	Females not receptive	X	X	Insect larvae

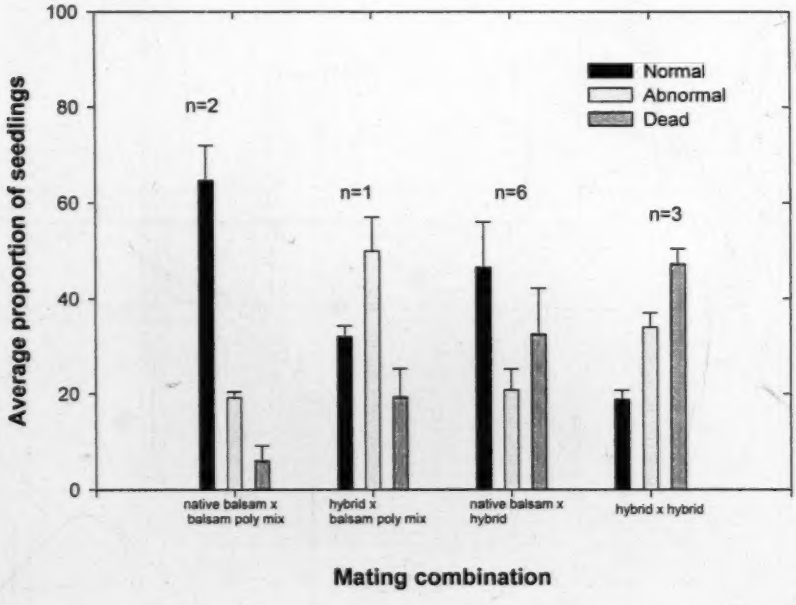
a. M = male parent in the first column, F = female parent in the first row.
b. See Chapter II (material and methods) for parentage of male and female hybrids.
c. Pb# = Native balsam poplar clones (1, 2, 3, 4).
d. Pb poly mix = pollen mix from 4 native *P. balsamifera* clones.

3.3.2. *Pollen viability and seed germination test*

All of the pollen lots, except for Brooks 6, produced a minimum of one pollen tube when tested for viability (Table 3-4). Seed was collected from 11 out of 30 crosses, a success rate of 40%.

The mating scheme between native x native produced the highest germination rate of normal seedlings (65%) relative to the proportion of abnormal (20%) and no seedlings (8%) compared to the other mating combinations (Figure 3-3). In the hybrid x hybrid mating combination, a higher proportion of the seed resulted in 45% of the seeds not germinating compared to the proportion of abnormal (35%) and normal seedlings (20%) relative to the other mating combinations (Figure 3-3). The mating combination between native x hybrid, on average, resulted in more normal seedlings (45%) than the reverse mating combination (30%) (Figure 3-3, see Appendix C for numerical values).

Figure 3-3. The average proportion of normal and abnormal seedlings, and seed resulting in no germination (across replicates and across each breeding combination) for each mating combination.



From the 11 (out of 30) successful crosses, native balsam x Brooks 1 (female parent always listed first) produced the most seed, while Russian x Manitou produced the least amount of seed. The three successful crosses with Manitou (male) all produced larger seed (in size) compared to the seed produced by the other crosses. Viable seed was collected from balsam (Pb#2) x Brooks 1, despite its negative pollen viability test results, and the cross between balsam (Pb#2) x Northwest was the only cross that produced 100% dead seed across all three replicates.

balsam x balsam: Seed from the cross Pb#3 x Pb poly-mix had a germination rate of approximately 80% normal seedlings, 20% abnormal seedlings, and 3% of the seed produced no seedlings (Figure 3-4A). The other female balsam (Pb#2) produced a smaller number of normal seedlings (45%), but a higher proportion of abnormal seedlings (41%) and dead seed (10%) (Figure 3-4A).

hybrid x balsam: There was only one successful cross from this breeding combination. The Russian x Pb poly-mix produced a higher proportion of abnormal seedlings (55%) compared to the number of normal seedlings (37%) and dead seed (20%) (Figure 3-4B).

balsam x hybrid: The two *P. balsamifera* x Manitou crosses exhibited extremely different results. The Pb#4 x Manitou produced the highest proportion of normal seedlings (93%) compared to the Pb#2 x Manitou cross (5%) (Figure 3-4B). The Pb#4 x Manitou cross had few abnormal seedlings (7%), whereas Pb#2 x Manitou had a higher proportion of abnormal seedlings (53%) and dead seed (42%) (Figure 3-4B).

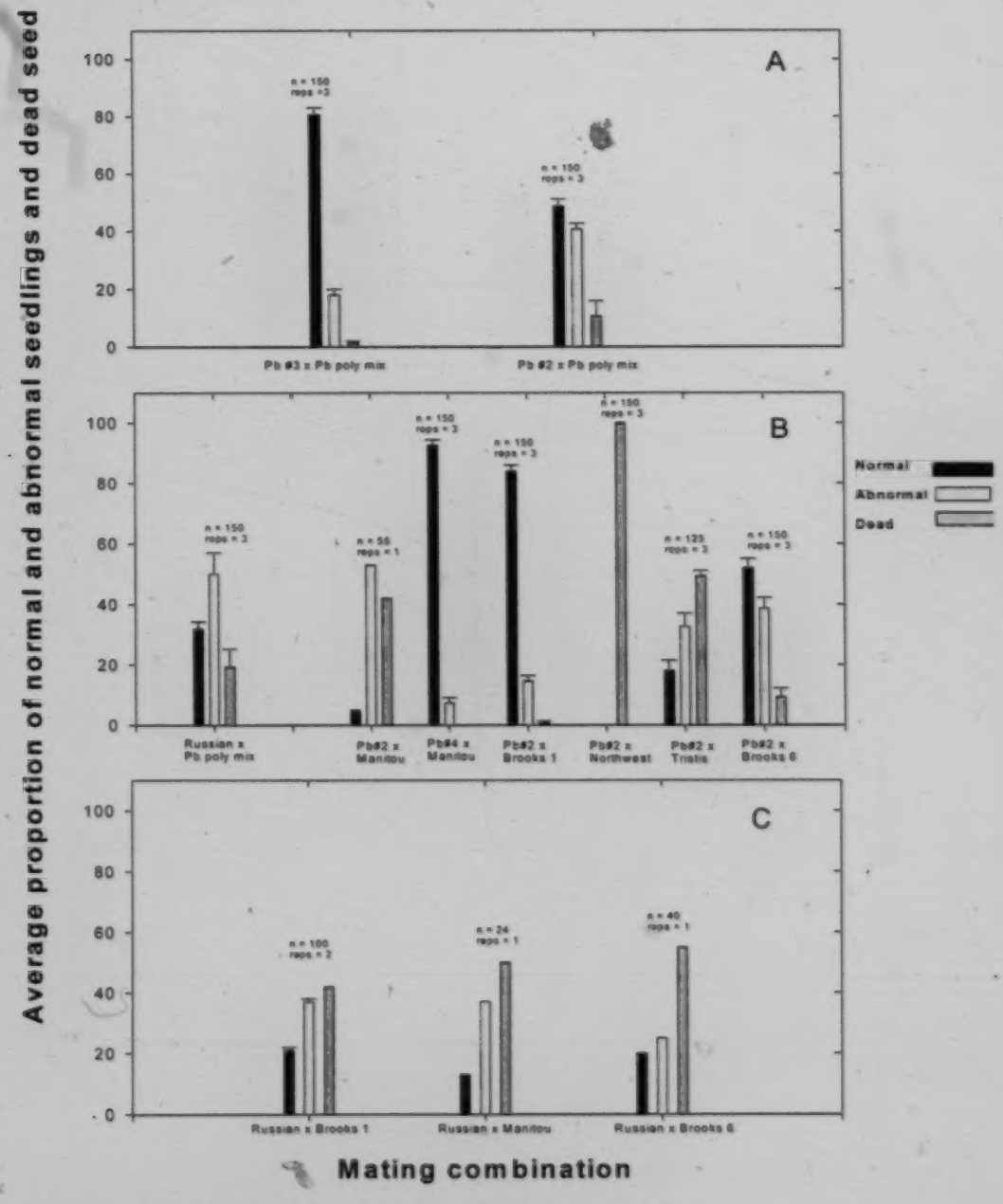
The cross Pb#2 x Brooks 1 also showed a high proportion of normal seedlings (84%) compared to 15% abnormal seedlings and an even smaller proportion of dead seed (1%). The cross Pb#2 x Brooks 6 produced a much lower number of normal seedlings compared to Pb#2 x Brooks 1, but it did produce a higher number of normal seedlings (53%) relative to the number of abnormal seedlings (39%) and dead seed (8%) (Figure 3-4B).

Seed produced from the single cross of Pb#2 x Tristis resulted in 49% of the seeds exhibiting no germination, 33% abnormal seedlings and only 18% normal seedlings (Figure 3-4B). Out of all the native x hybrid crosses the seed from Pb#2 x Tristis and Pb#2 x Manitou resulted in fewer normal seedlings compared to the other crosses of this mating combination (Figure 3-4).

hybrid x hybrid

The Russian x Brooks 1, Brooks 6, and Manitou crosses exhibited normal seedling germination rates of 21%, 20%, and 13%, respectively, and rates of 37%, 25%, and 37% for abnormal seedlings (Figure 3-4C). On average, the seed from these three crosses produced a higher percentage of dead seed (42%, 55%, 50% respectively) than the development of normal and abnormal seedlings (Figure 3-4C).

Figure 3-4. The average proportion of normal and abnormal seedlings, and seed resulting in no germination (across replicates) for each individual breeding cross for each combination (female x male): A) native x native, B) hybrid x native and native x hybrid, C) hybrid x hybrid.



1. Pb #2, 3, 4 are different female balsam clones used in the breeding experiment.
2. Pb poly-mix is a mixture of pollen from four balsam poplar clones.

3.4. DISCUSSION

3.4.1. *Seedling variability*

Overall, the mating combination between native x native balsam produced a higher proportion of normal seedlings relative to the number of abnormal seedlings, in contrast to those produced in the other mating combinations. Some researchers believe that the mating combinations between F_1 female hybrids and either native male species or male hybrids are expected to show reduced fitness (Rosalind et al. 1998), which could ultimately limit their potential to exchange genes with native species. More specifically, studies have shown some crosses between female hybrid and native male poplars yield more viable seed than the reverse mating combination (native female x hybrid male) (Strauss and DiFazio 1997). In this study, we only had one successful cross between female hybrid x native male balsam, in contrast to the six successful crosses from the female balsam x male hybrids of varying results; therefore, it is difficult to compare which mating combination could potentially produce more abnormal seedlings.

In this study, a wide range in normal seedlings (49% to 81%) was observed between the two balsam x balsam crosses (using two different female clones with the same balsam poly-mix pollen) (Table 3-3 and 3-4A). This variability among native crosses shows how individual female clones within a native population may play a role in obtaining either success or failure in any given cross, demonstrating genetic variability among poplar clones.

This wide range of variability among normal seedlings was also observed in the mating combinations between native females x hybrid males (*P. balsamifera* x Northwest = 0% to *P. balsamifera* x Manitou = 93%), and the hybrid x hybrid crosses (Russian x Manitou = 13% to Russian x Brooks 1 = 21%).

3.4.2. *Occurrence of premature capsule abscission*

The occurrence of premature capsule abscission observed in 12 of the 30 crosses may have been a function of 1) the lack of a functioning root system, 2) pre-fertilization and/or post-zygotic barriers, and 3) hybrid inviability.

1) Some branches appeared to not have a functioning root system since leaf growth ceased shortly after emergence. This lack of a functioning root system could

have resulted in capsule abscission, as there might not have been enough energy to support the long seed development phase. However, the amount of fine root hairs required to support the seed development phase is unclear as branches with good seed production had the same number of root hairs as those observed on the branches expressing premature capsule abscission. In addition, these same female branches broke bud on day 14 compared to the typical 21 or 28 days, which is during the same time period when roots should have been developing. Some of these females could have had an adverse reaction to the light levels being too high during this period as some protocols suggest female branches should be kept in the dark during this phase (Dan Walker, Per. Comm. 2001). Another factor that could have contributed to early capsule abscission was the presence of a few vegetative buds. Prior to the application of pollen, most vegetative buds were removed. However, Dan Walker, who follows a similar protocol, experienced similar breeding results, and commented that he will omit the pruning of vegetative buds in the 2002 breeding project.

2) Premature capsule abscission is associated with both pre-fertilization and post-zygotic barriers (Stettler et al. 1996). Pre-fertilization barriers are commonly described as the dysfunction of pollen tubes whereby the tube does not elongate and fertilize receptive ovules, and post-zygotic barriers are thought to be linked to the disruption of embryo maturation through premature capsule abscission or the development of empty seed (Stettler et al. 1996). In this study, early capsule abscission might have been associated with post-zygotic barriers rather than pre-fertilization barriers, as all the crosses had developing green fruits, suggesting that pollen tubes were functioning and female ovules fertilized. Post-zygotic barrier refers to embryo abortion, which may have been responsible for the development of empty seed produced by the balsam x Northwest cross, as a lot of seed was produced, but no seed germinated.

3) The third reproductive barrier known as 'hybrid inviability' refers to the weak growth of seedlings during the first few months or years, and performance progressively declining over time (Stettler et al. 1996). This reproductive barrier is not often referenced as researchers generally report successful breeding crosses with the production of seed (Zsuffa et al. 1999). However in this study, the observation of a high number of abnormal seedlings might be associated with 'hybrid inviability'. The host of

reproductive barriers are not well understood; therefore, they provide a series of alternative explanations to help us understand the complex process of reproduction, but it is believed that reproductive barriers become more complex in preventing the production of viable seed, as the process moves from pre-fertilization to post-zygotic barriers to hybrid inviability (Stettler et al. 1996).

3.4.3. *Summary and conclusion*

The objective of the study conducted in this chapter was to determine if native and non-native poplars are genetically compatible under greenhouse conditions by conducting a series of breeding experiments and determining if seed could be produced and then assessing the viability of that seed. If hybrid seed could be produced in the greenhouse, then hybrid seed might also be produced naturally if all the environmental conditions are met. From the 30 crosses, we expected to collect seed from each of the four mating combinations (pure balsam and hybrid crosses) with an overall success rate of 50%. Seed was collected from 12 of the 30 crosses (a success rate of 40%) and from each mating combination; however, there was no seed collected from the female hybrid clone Walker. The failed crosses either exhibited premature capsule abscission, dead seed, or insect larvae feeding on the developing seed. Therefore, it is not possible to conclude whether post-zygotic barriers or the lack of a functioning root system were responsible for the premature capsule abscission in 12 of the 30 crosses. However, one can conclude that since seed was produced from at least one of each of the possible mating combinations (native x native; native x hybrid; hybrid x native; hybrid x hybrid), suggesting that under the right environmental conditions, hybrid seed can be produced from both native female *P. balsamifera* clones and introduced hybrid female poplars. The next step is to determine whether hybrid seed is being produced naturally.

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CHAPTER FOUR: Determination of Putative Hybrids Based on Leaf Morphology

4.1. INTRODUCTION

4.1.1. *Background*

In the previous two chapters, it was suggested that hybridization between native and non-native poplars is possible based on the observed flowering patterns and the proportion of normal hybrid seedlings obtained from the greenhouse breeding experiment. Therefore, if all environmental factors were met, it can be possible to determine if hybridization is occurring naturally, by examining leaf morphology of seedlings produced by seed collected from both native and non-native trees adjacent to native and hybrid male trees. Past studies have shown that leaf morphology can be used as a diagnostic feature for the identification of poplar species within and between taxonomic sections and for the identification of naturally occurring hybrid poplars (Barnes 1969; Barnes 1975; Vanende 1990). However, there is tremendous variation in leaf morphology due to poplar's heterophyllous growth habit, where two types of leaves (early and late) on two types of shoots (determinate and indeterminate) are produced, and the proportion of both types of leaves and shoots are dependent upon the age of the tree, location within the crown, and the growing season (Critchfield 1960). Therefore, leaf size and shape can vary within a crown and along a shoot, requiring a standardized protocol for the collection of leaves to make consistent leaf assessments (Barnes 1969).

4.1.2. *Heterophyllous development*

Within each series of early and late leaves there are differences in leaf size, and between the two series of leaves there are differences in both leaf size and shape (Eckenwalder 1996). Determinate growth (short shoot) produces early leaves, and indeterminate growth (long shoot) produces both early and late leaves (Critchfield 1960). Early leaves are the first set of leaves to flush in the early Spring, originating from buds formed in the previous growing season (Critchfield 1960). During the first two weeks of bud flush it is difficult to distinguish between determinate and indeterminate shoots, but after a few weeks of growth, determinate shoots cease growth and develop a terminal bud. Indeterminate shoots continue to grow and elongate throughout the growing season,

producing late leaves. Determinate shoots produce five to seven early leaves with no stem elongation, and indeterminate shoots produce approximately seven early leaves with little stem elongation, followed by one or two transition leaves, and then growth continues throughout the growing season, producing a series of late leaves (Critchfield 1960). The number of late leaves produced depends on the environmental conditions during a growing season (Critchfield 1960).

Within the early-leaf series in both determinate and indeterminate shoots, the first and second leaves are typically smaller than the other leaves (Critchfield 1960). In long shoots, the transition leaves between early and late leaves are usually smaller than both the early and late leaves, and late leaves are typically larger and more variable in shape than early leaves, (Critchfield 1960; Barnes 1969). It is also believed that because early leaves are formed in the bud (during the previous growing season) they are under moderate to strong genetic control, and they are less likely to be influenced by environmental factors (Critchfield 1960; Barnes 1966; Dancik and Barnes 1975).

The proportion of determinate and indeterminate shoots is directly related to the age of the tree. Young trees produce more indeterminate shoots than determinate shoots, which contribute to the development of the crown. However, in mature trees with developed crowns, there are more determinate shoots than indeterminate shoots, and indeterminate shoots are typically found in the upper and outer crowns (Critchfield 1960).

4.1.3. *Objective*

In this study, the objective was to determine if hybridization between native and non-native poplars is occurring naturally by assessing leaf size and shape of progeny from open-pollinated native balsam and hybrid poplar clones adjacent to native balsam and/or hybrid male poplar clones. Open-pollinated (o.p.) seeds (known female parent and putative male parent(s)) and seeds from the controlled crosses of known parentage (Results, Chapter three) were sown in the greenhouse. After a five-month growing period, early leaves were collected from all seedlings, and a series of leaf characteristics were measured and scored for leaf size and shape. Early leaves were also collected from the parents of the o.p. seeds to compare their leaf measurements with those of the seedlings.

Leaf ratios were calculated and graphed in excel, followed by a multivariate and discriminate analysis. These analyses were used to look for discrete groupings of the mature hybrid and balsam clones with overlapping clusters of progeny from the open-pollinated and controlled crosses of similar parentage.

4.2. MATERIAL AND METHODS

4.2.1. *Seed collection and extraction, and seedling development*

On June 24, 2000, seed capsules were collected from four native balsam and five non-native (Russian poplar clones from four different locations and Walker from one location) poplar clones, which were adjacent to both native and non-native male poplar clones (Table 4-1). During the field collection, seed capsules were placed in small, labeled plastic bags, brought to the University of Alberta, and stored in a cooler (4^0C) for one day. The following day, seed capsules were placed between two 60cm x 60cm mesh screens (one seedlot between two mesh screens) at room temperature for further ripening and release of white cotton fibers. After four-days of air-drying, seed was extracted from the white cotton fibers, tested for viability, and stored in glass vials at -23^0C in a non-frost-free freezer to prevent exposure to fluctuating temperatures. As in chapter three, the seed germination test was done following the procedure described by Edwards (1987), which recommends using 100 seeds per replicate and four replicates where possible. However, due to the limited number of seed, viability tests consisted of 50 seeds per seedlot and four replications.

Table 4-1. Female parent from which open pollinated seed was collected, distance to putative male parents and location area of seed collection.

Female parent	Possible male parents, and their approximate distance from the female parent	Location
Balsam	Balsam, 15 m north Brooks 1[a], 50 m east	Athabasca
Balsam	Balsam, 100 m west Northwest, 1 km north	Ft. Saskatchewan
Balsam	Balsam, 30 m east Brooks 1, 10 m north	Ft. Saskatchewan
Balsam	Balsam, 5 m east and west Northwest, 50 m north	Ft. Saskatchewan
Russian	Balsam, 30 m northwest Brooks 6, 2m east	Athabasca
Russian	Balsam, 10 m north	Athabasca
Russian	Balsam, 1 km north and west Northwest, 30 m north	Ft. Saskatchewan
Russian	Balsam, 500m surrounding area Brooks 6, 50 m south	Ft. Saskatchewan
Walker	Balsam, 30 m north and west Manitou, 10 m west	Athabasca

a = For parentage of male and female hybrid clones see Chapter II, Material and Methods.

In October 2000, open-pollinated seeds were sown in Hillson root trainer trays (100 cavities, Type 170-4. Spencer-Lemaire Industries, Edmonton, Alberta) and placed in a greenhouse with a 16-hour photoperiod and a diurnal temperature of 24^{0}C (day) and 17^{0}C (night). A fungicide drench (Truban 0.33ml/L. Mallinckrodt Canada, Pointe Claire, Quebec) was applied once in November and again in December to control damping off. In February, Pentac (0.65 ml/L. Sandoz Agro Canada Inc., Mississauga Ontario) was applied to control spider mites and Trupet (0.5gm/L. Plant Products. Bramalea, Ontario) was applied for the control of thrips. Both of these products were re-applied 10 days later to try and break the life cycle of both pests.

In order to collect and measure early leaves, the seedlings needed to set bud, and re-flush, developing long shoots (both early and late leaves), as the leaves produced from first year seedlings are considered late leaves, developing from existing primordia tissue and not from a dormant bud (Isebrands and Larson 1973). After five months of growth, in the greenhouse, seedlings set bud and ranged between 30 and 50cm in height. By pinching off the terminal bud of each plant, lateral buds flushed and produced early leaves on long shoots within three weeks.

4.2.2. *Leaf collection and measurements*

A standardized protocol for collecting leaves from mature trees was used to help eliminate sampling bias and leaf heterophylly found within *Populus* trees. For the known mother and putative fathers, early leaves borne on short shoots from the lower crown and along the mid-section of the main branch (Barnes 1966; Barnes 1969; Barnes 1975) were collected and measured. In order to capture intra-clonal variation expressed in *P. balsamifera* leaves were collected from 10 male and 10 female clones.

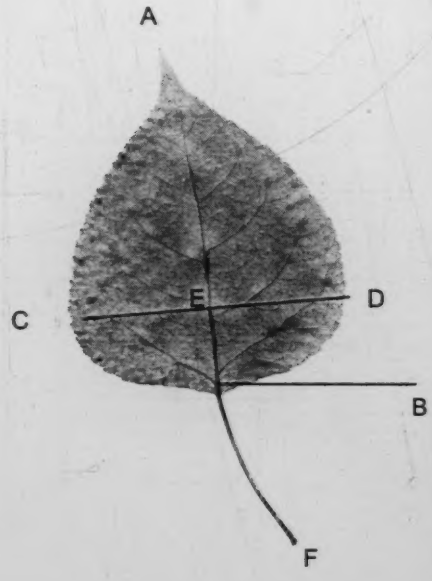
In October 2001, the seedlings generated from the controlled crosses (c.c.) described in Chapter III, set bud (in the greenhouse). In order to collect and measure early leaves, the seedlings needed to set bud and have a period of cold dormancy. Therefore, during the month of November, greenhouse conditions were changed from a 16-hour photoperiod to a natural (ambient) photoperiod. In early December, plants were moved to a dark room and held at 8^0C. After 14 days, plants were moved into an insulated and heat controlled garage for cold storage. In the garage, natural light was blocked out, plants were raised off the ground, and the air temperature varied between 3^0C and -8^0C (depending on the outdoor temperature) for seven weeks. On January 28, 2002 plants were brought back into the greenhouse to induce bud flush.

After three weeks, two early leaves (generally, leaf numbers three and five from the shoot base) were collected from long shoots, then measured and pressed from all o.p. and c.c. seedlings, and from mature trees. Only one leaf was used for leaf measurements (the second leaf was kept as a back-up), as it was previously determined by Barnes (1969) that the morphological features of one leaf per clone is sufficient to capture intra- and inter-clonal variation. All leaves were measured and scored for six morphological characteristics (Figure 4-1) (Barnes 1966; Barnes 1969; Barnes 1975). Methodology in Harris and Harris (2000) was followed to characterize (or score) leaf shape (leaf base and apice).

Figure 4-1. Diagram of a poplar leaf showing measurement points for morphological features for A) whole leaf, B) leaf base, C) leaf apice.

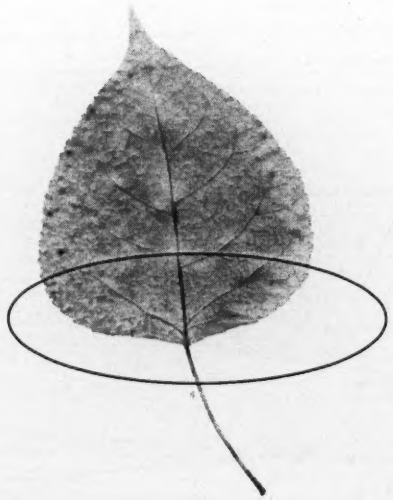
A. Morphological features measured for all leaves (absolute values measured in mm):

1. Blade length (AB);
2. blade width (CD);
3. position of maximum width (BE);
4. petiole length (BF);



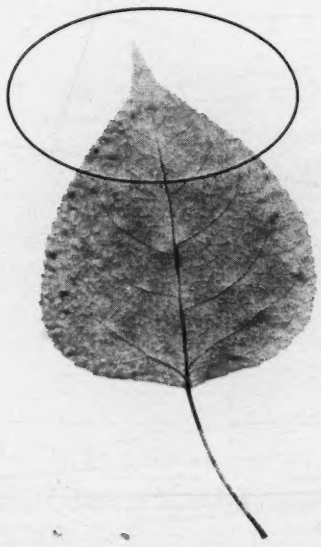
B. Morphological features characterizing leaf base. Description of terminology can be found in Harris and Harris (2000).

1. cordate
2. truncate
3. round
4. aequilateral
5. cuneate



C. Morphological features characterizing leaf apice. Description of terminology can be found in Harris and Harris (2000).

1. cuspidate
2. acuminate
3. acuminate - aristulate
4. aristulate
5. aristulate - acute
6. acute
7. acute - subacute
8. subacute
9. subacute - round
10. round



4.2.3. *Assessment of leaf morphological measurements*

Initially, the ratio of petiole length versus blade length (P/L) was calculated using excel (Microsoft Office 2000) and plotted against the ratio of blade width versus blade length (W/L), and the ratio of position of the maximum blade width versus blade length (M/L) was plotted against the ratio of blade width versus blade length (W/L) for each of the open pollinated (o.p.) seedlings, known female parent and putative male parents to determine the proportion of hybrid and non-hybrid seedlings. However, there was no clear relationship between the mature leaves and the leaves collected from the one-year-old o.p. seedlings. Therefore, leaves collected from the controlled crosses of known parentage (Results, Chapter III) were measured and analyzed with the o.p. seedlings of similar parentage. Due to the small number of seedlots from the controlled crosses sharing similar parentage as the o.p. seedlings only six o.p. seedlots were analyzed (Table 4-2).

For each open pollinated seedlot combination (described in Table 4-2), the ratios P/L against W/L, and the ratios M/L against W/L were plotted. For example, individual plots consisted of leaf measurements from a) seedlings from the controlled crosses of similar parentage, b) female parent (and other female balsam clones), and the putative male parents of the o.p. seedlings, and c) o.p. seedlings sharing similar parentage as the seedlings of the controlled crosses.

Table 4-2. Female parent x putative male parent breeding cross of open pollinated seedlings, location of female parent, and parentage of controlled cross seedlings.

Female parent x putative male parents	Location of female parent tree (landowner)	Controlled crosses of known parentage
Russian[1] x Balsam, Northwest	Jeske	Russian x Balsam
Russian x Balsam	Daniels	Russian x Balsam
Russian x Balsam	Shymoniak	Russian x Balsam
Russian x Balsam, Brooks 6	Brytus	Russian x Balsam Russian x Brooks 6
Balsam x Balsam, Brooks 1	Lameraux	Balsam x Balsam Balsam x Brooks 1
Balsam x Balsam, Brooks 1	Chursch	Balsam x Balsam Balsam x Brooks 1

1. Female x male (female parent always listed first).

In an attempt to simplify any relationships possibly observed by the overlapping of several pairs of variables, a multivariate approach was used, using principal component (PC) and canonical variate (CA) analysis. Both PC and CV analysis have been used widely in studies of populations to portray differences among groups when more than one variable is measured (Barnes 1969; Barnes 1975; Dancik and Barnes 1975; Barnes and Han 1992). In both of these programs, absolute values were used.

Principal component (PC) analysis

PC analysis derives a linear combination of variables that explains the maximum variation between individuals regardless of parentage or origin (Reyment et al. 1984; James and McCulloch 1990). The number of computed principal components is equal to the number of variables. The first principal component accounts for the maximum variation present in multivariate space, and can be thought of as the largest vector that can be passed through the data in multi-dimensional space (Reyment et al. 1984). The second component accounts for the largest proportion of the remaining variation.

Principal components were plotted in simple scatter plots. The position of individual c.c. and o.p. seedlings was compared with the position of known female and putative male parent(s). The PC analysis was re-run without the parents to compare the position of individual seedlings, representing the progeny from the controlled crosses with the o.p. progeny of similar parentage. Analysis was completed using PRINCOMP (SAS, version 8.0) to compute the principal components.

Canonical discriminate variate (CV) analysis

CV analysis also derives a linear combination of variables that explains the maximum variation between known groups of individuals (identified prior to the analysis), relative to the variation within each group. The first canonical variable (CV1) is a linear combination of the variables that provides the greatest difference between group means (Reyment et al. 1984). The second canonical variable (CV2) is perpendicular to CV1 and is also a linear combination of variables, maximizing the next greatest variation between group means while being uncorrelated with the first canonical variable.

Canonical variables were plotted in simple scatter plots, displaying the position of points from the unknown group identified as putative hybrids relative to the position of

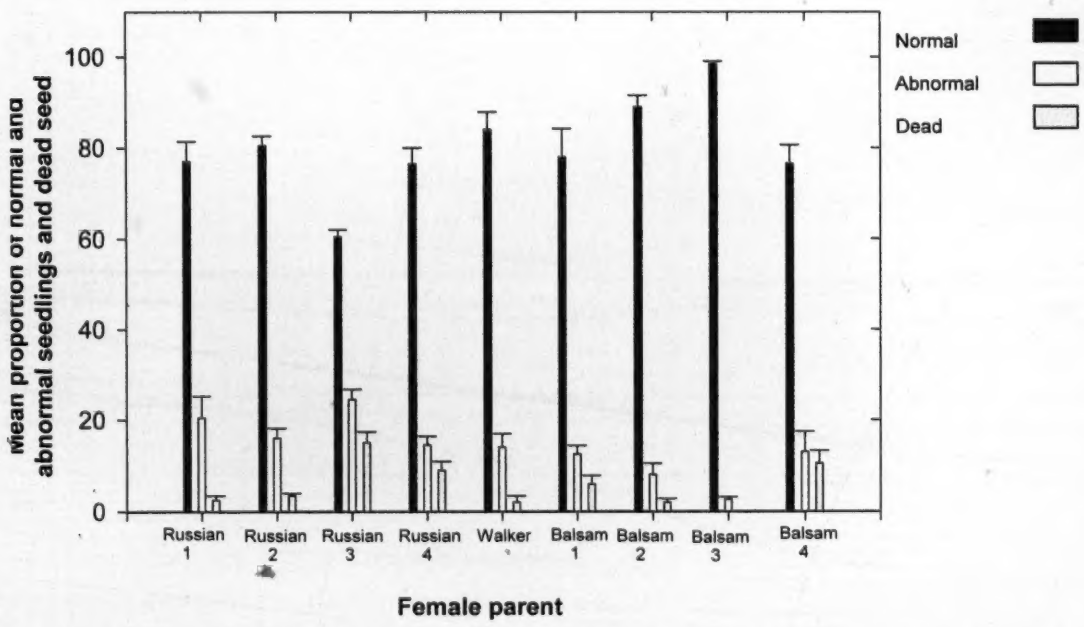
individual points from known groups (controlled crosses). Analysis was completed using CANDISC (SAS, version 8.0) to compute the canonical variates.

4.3. RESULTS

4.3.1. *Seedling variability*

The open-pollinated seed collected from two female hybrid poplar (Russian from four different locations, and one Walker) and four native balsam clones exhibited a relatively high proportion of normal seedlings (60.5% to 98%) with a corresponding low proportion of abnormal seedlings (8% to 24%) (Figure 4-2). In terms of the proportion of normal and abnormal seedlings, there is little variation between progeny from the two female hybrid poplars (Walker and Russian) and native female balsam clones.

Figure 4-2. The average proportion of normal and abnormal seedlings and seed resulting in no germination (across replicates) from open pollinated female hybrids and female balsam poplars (located in Ft. Saskatchewan and Athabasca).



Location of trees: Russian 1, 2, 3, 4, consecutively, Jeske, Shymoniak, Daniels, and Brytus. Balsam 1, 2, 3, 4, consecutively, Jeske, Lamoureux road across from Northwest, Lamoureux road across from Brooks 1, and Chursch.

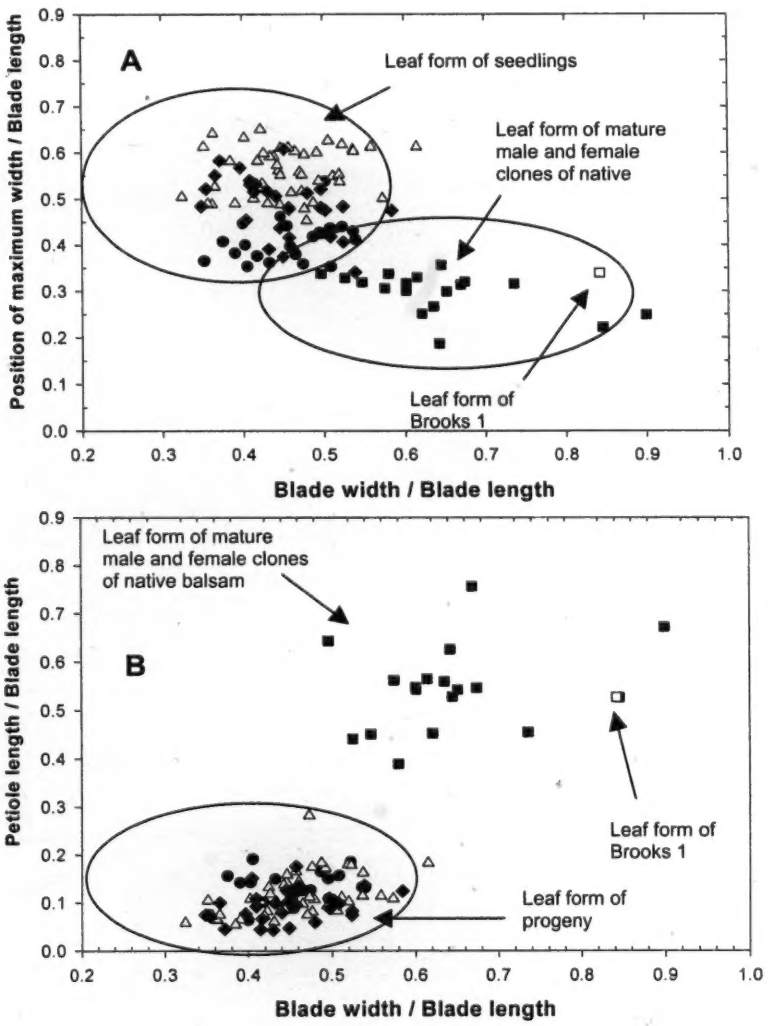
4.3.2. *Visual observations of leaf characteristics*

While collecting leaves, differences in leaf size and shape were apparent between the mature trees and one-year-old seedlings. Mature leaves (parents) were larger in size with a distinctive shape compared to the seedling leaves. Leaf serrations in the mature trees were distinct, ranging from small round teeth margins found in native balsam to large round teeth margins found in the hybrid poplar clones. Leaf shape was also distinct, ranging from cordate to round. Other differences such as red petioles and wavy leaf blades, found only in Brooks 1 were also noted. However, leaf size and shape were similar among all seedlings, regardless of parentage. Leaves from one-year-old seedlings were juvenile, displaying small leaf blades, short petioles, no leaf serrations, and similar leaf shape among seedlings, regardless of parentage.

4.3.3. *Leaf ratio assessment*

Plotted leaf ratio of petiole length versus blade length (P/L) against blade width versus blade length (W/L) was effective in separating the mature leaves from the juvenile leaves, in comparison to the plotted ratio of the position of maximum width versus blade length (M/L) against the ratio of W/L (Figure 4-3). Leaf ratios from juvenile leaves of different seedlots (o.p. crosses and controlled crosses) exhibited no discernible pattern of clusters, only continuous variation among seedlings, regardless of parentage (Figure 4-3).

Figure 4-3. Leaf ratios of mature male and female tress of *P. balsamifera* (■), Brooks 1 (putative father □), open pollinated seedlings from female balsam parent (●), and controlled cross seedlings from known parentage (balsam x balsam ◆ , and balsam x Brooks 1 △) from the greenhouse breeding experiment (Chapter three) for A) position of maximum width/blade length versus blade width/blade length, and B) petiole length/blade length versus blade width/blade length.


A
Leaf form of seedlings
Leaf form of mature male and female clones of native
Leaf form of Brooks 1
Position of maximum width / Blade length
Blade width / Blade length
B
Leaf form of mature male and female clones of native balsam
Leaf form of Brooks 1
Leaf form of progeny
Petiole length / Blade length
Blade width / Blade length

Circled areas denote the groups of juvenile and mature leaf form.

4.3.4. *Multivariate analysis*

Principal component analysis

Results graphed from the principal component (PC) analysis showed similar patterns of clustering as observed in the plotted leaf ratios. Foliar analysis for each of the six open-pollinated (o.p.) progenies with the controlled (c.c.) crosses of similar parentage (listed in Table 4-2) showed a clear separation between mature and juvenile leaves, but there was no clear separation of the different seedlots based on the principal component axes. The open-pollinated progeny from native balsam adjacent to male balsam and Brooks 1 (putative male parents located in Athabasca) is shown in Figure 4-4, illustrating clear separation between mature and juvenile leaves (only one graph is presented, as the results were similar for all PC runs). However, there was no clear separation between the data points representing seedlings of the c.c. and o.p. seedlots (Figure 4-4). The most heavily weighted variables (from the six measured variables) contributing to the first principal component (axis 1) were blade width and petiole length (Table 4-3). These two leaf characteristics clearly separate the mature leaves from young seedling leaves, whereby mature leaves have wider leaf blades and longer petioles. The second principal component (axis 2) was largely related to the position of maximum width and petiole length (Table 4-3). The first two principal components from the six original variables accounted for 77% of the total variation (Table 4-4).

To observe more separation between the different groups of progenies (controlled crosses and open pollinated), known female parent and putative male parents of the o.p. progeny were removed from the data set. Results from the PC analysis showed slightly more separation between the c.c. and o.p. seedlots (again only one graph is presented as all PC runs without parents were similar), but there was no clear separation between the two known crosses (controlled crosses) (Figure 4-4). The o.p. progeny had the widest variation compared to both of the controlled crosses (balsam x balsam, and balsam x Brooks 1), and individual points from all three groups overlapped (Figure 4-5). Both blade width and length were the most heavily weighted variables for the first axis, and both base and apice shape were the most heavily weighted variables for the second axis, accounting for 70% of the total variation in the first and second axis (Table 4-5 and 4-6).

Figure 4-4. Principal component analysis (using the first and second axis) of leaf form based on six morphological features measured for the open pollinated (o.p.) seedlings from native female balsam (■), controlled cross seedlings of balsam x balsam (▲) and balsam x Brooks 1 (●), and mature trees from both female and putative male parents (female *P. balsamifera* clones and male *P. balsamifera* clones (▼) and Brooks 1 (◆)). Circled area denotes leaf form of mature balsam and hybrid (Brooks 1) poplar clones.

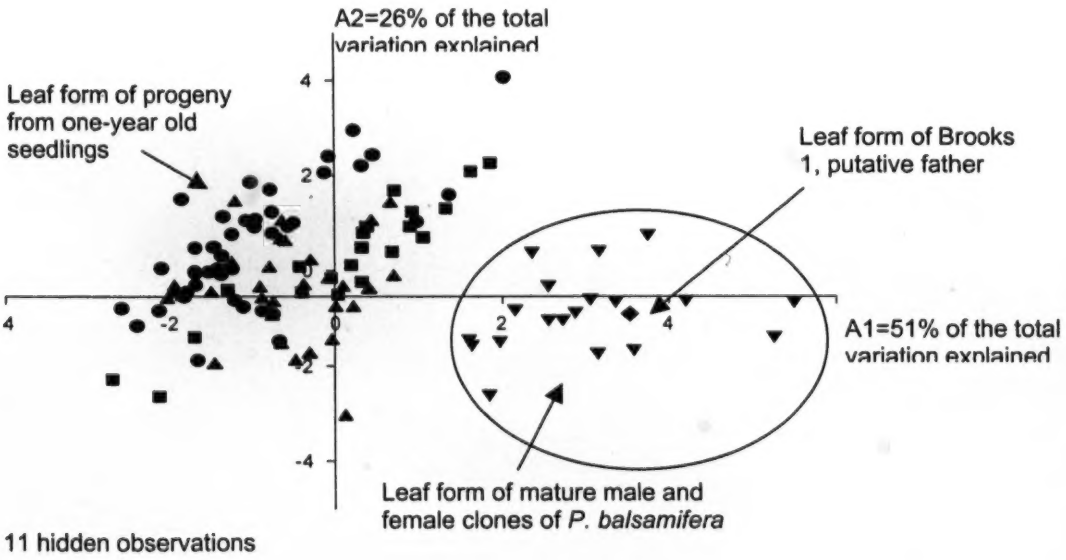

A2=26% of the total variation explained
Leaf form of progeny from one-year old seedlings
Leaf form of Brooks 1, putative father
A1=51% of the total variation explained
Leaf form of mature male and female clones of P. balsamifera
11 hidden observations

Figure 4-5. Principal component analysis (using the first and second axis) of leaf form based on six morphological features measured for the open pollinated (o.p.) seedlings from native female balsam□), and the controlled cross seedlings from balsam x balsam (▲) and balsam x Brooks 1 (●).

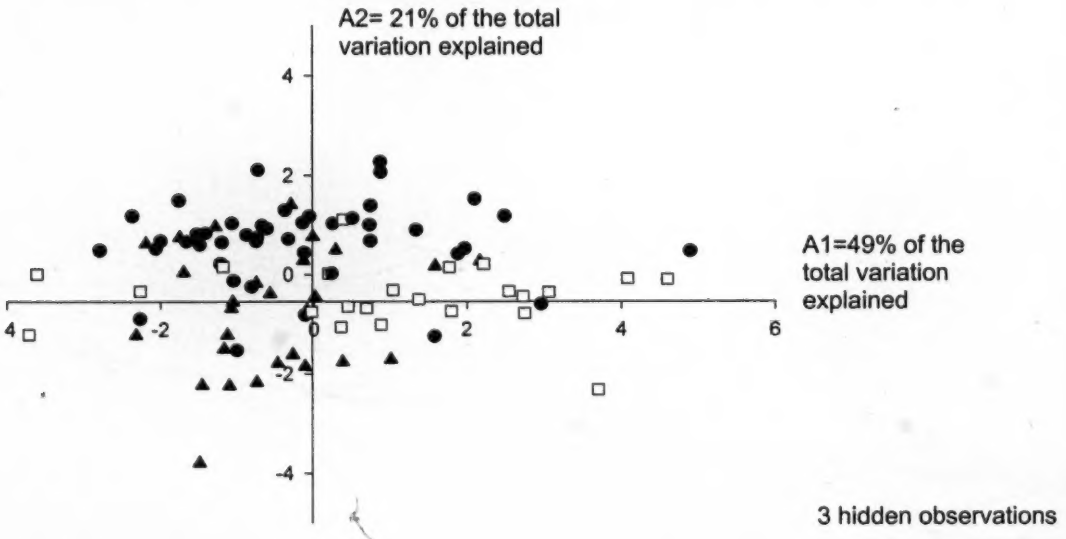


Table 4-3. Leaf variables (four measured and two derived) and associated weightings for the first two principal components (A1 and A2) of a principal component analysis of open pollinated (o.p.) and controlled cross seedlings of known female (of o.p. seedlings) and putative male poplar parents (native balsam clones and Brooks 1).

	Weighting	
Variable	**Component 1 (A1)**	**Component 2 (A2)**
Blade length	0.446	0.462
Blade width	0.532	-0.140
Position of maximum width	0.019	0.764
Petiole length	0.489	-0.191
Leaf base shape	-0.369	-0.297
Leaf apice shape	-0.377	0.243

Table 4-4. Principal component, eigenvalues, proportion of the explained variation for each eigenvalue, and cumulative variation explained from the principal component analysis with the known female and putative male poplar parents.

Principal component	**Eigenvalue**	**Proportion**	**Cumulative**
1	3.070	0.5112	0.512
2	1.572	0.262	0.774
3	0.624	0.104	0.878
4	0.545	0.091	0.969
5	0.118	0.020	0.988
6	0.071	0.012	1.000

Table 4-5. Leaf variables (four measurred and two derived) and associated weightings for the first two principal components of a principal component analysis of open pollinated progeny from female balsam adjacent to native balsam and Brooks 1, and controlled cross seedlings (balsam x balsam, balsam x Brooks 1).

	Weighting	
Variable	**Component 1 (A1)**	**Component 2 (A2)**
Blade length	0.559	0.055
Blade width	0.554	-0.002
Position of maximum width	0.443	0.382
Petiole length	0.391	-0.100
Leaf base shape	-0.102	0.632
Leaf apice shape	-0.144	0.665

Table 4-6. Principal component, eigenvalues, proportion of the explained variation for each eigenvalue, and cumulative variation explained from the principal component analysis (without parents).

Principal component	Eigenvalue	Proportion	Cumulative
1	2.941	0.49	0.49
2	1.242	0.21	0.70
3	0.807	0.13	0.83
4	0.768	0.13	0.96
5	0.164	0.03	0.99
6	0.077	0.01	1.00

Canonical discriminate variate analysis

The graphical display of the canonical variables for each of native balsam by hybrid male, and hybrid female by native balsam were similar, therefore, the results from the balsam by Brooks 1, and the Russian by balsam data combinations are shown in Figures 4-6 and 4-7.

In contrast to the principal component analysis, the graph results of the first two canonical variables showed a wider separation among individuals within a group, and grouping of the three different progenies (two known sets of progeny from the controlled

crosses, and one open pollinated progeny set). The plot of the first two canonical (Can) variables for the balsam by Brooks 1 data set shows that Can1 discriminates between all three progeny groups: open-pollinated balsam, and controlled crosses balsam x balsam and balsam x Brooks 1 (Figure 4-6). Can 2 best discriminates between open-pollinated balsam and the control cross balsam x balsam progeny (Figure 4-6). The first canonical variable accounted for 74% of the total variation explained. Similarly, the plot of the first two canonical (Can) variables for the Russian by balsam data set shows that Can1 discriminates between all three progeny groups: open-pollinated Russian, and the controlled cross of Russian x balsam and balsam x balsam (Figure 4-6). Can2 best discriminates between open-pollinated Russian and the control cross balsam x balsam progeny (Figure 4-7). The first canonical variable accounted for 71% of the total variation explained (Figure 4-7).

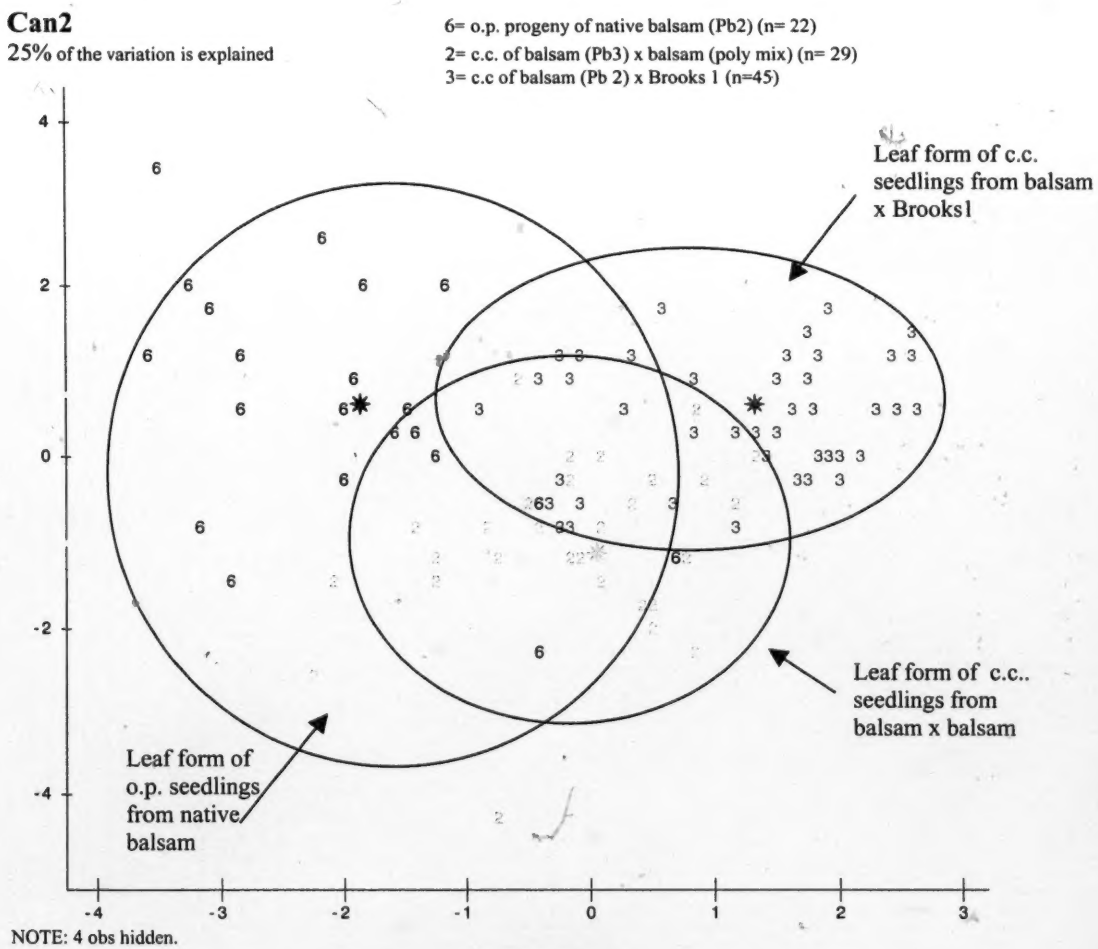
The putative male parentage of the open-pollinated progeny of both balsam and Russian exhibited a wider distribution than either of the two known groups of progeny from the controlled crosses. However, several individual points from all three groups do overlap in both the balsam by Brooks 1, and the Russian by balsam data combinations.

The coordinates of the group means are presented in Figure 4-6 and 4-7. SAS computed the Mahalanobis distance, which determines whether the mean distance between groups are significantly different. In the balsam by Brooks 1 data combination, all three groups are significantly different from each other ($p < 0.0001$), even though individuals within each group overlap. In the Russian by balsam data combination, the three groups are also significantly different ($p<0.0001$), although the two known groups of progeny (balsam x balsam, and Russian x balsam) are significantly different at the $p = 0.02$ level. The multivariate test for differences between the groups of progeny is also significant at the $p = 0.0001$ level for both the balsam by Brooks, and Russian by balsam data set combinations.

The raw canonical coefficients for the first and second canonical variables show that the groups of progeny differ most widely on the linear combination of the centered variables (Table 4-7). In the balsam by Brooks data set, leaf apice and position of maximum width are the variables contributing the most variation in the linear

combination of centered variables of Can1, whereas in the Russian by balsam data set, it was leaf base and leaf apice (Table 4-7).

Figure 4-6. Plot of the first and second canonical discriminate variables based on the six morphological leaf characteristics measured for open pollinated (o.p.) seedlings from native female balsam (located at Chursch in Athabasca) and controlled cross seedlings (c.c.) of balsam x balsam, balsam x Brooks 1. Can1 has the most discriminatory power.


Can2
25% of the variation is explained
6= o.p. progeny of native balsam (Pb2) (n= 22)
2= c.c. of balsam (Pb3) x balsam (poly mix) (n= 29)
3= c.c of balsam (Pb 2) x Brooks 1 (n=45)
Leaf form of c.c. seedlings from balsam x Brooks1
Leaf form of c.c.. seedlings from balsam x balsam
Leaf form of o.p. seedlings from native balsam
NOTE: 4 obs hidden.
Can1
75% of the variation is explained

✱ = coordinates of population mean of balsam x Brooks 1 (1.059, 0.397)
✱ = coordinates of population mean for balsam x balsam (-0.164, -1.0378)
✱ = coordinates of population mean for o.p. balsam (-1.950, 0.556)

Figure 4-7. Plot of the first and second canonical variables of the six morphological leaf characteristics measured for open pollinated (o.p.) seedlings from female hybrid Russian (located at Daniels in Ft. Saskatchewan), and controlled cross seedlings of balsam x balsam, balsam x Brooks 1. Can1 has the most discriminating power.

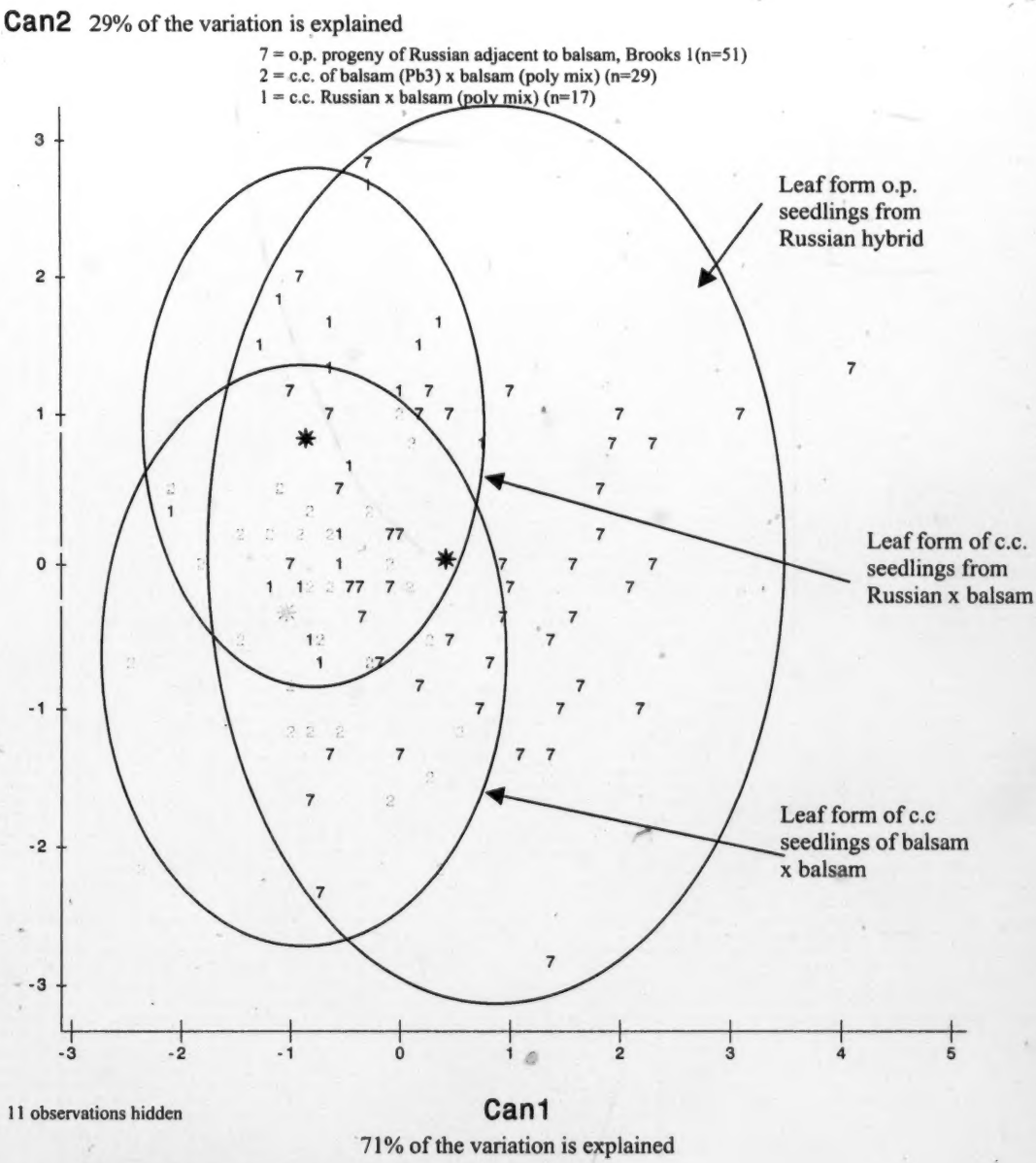

Can2 29% of the variation is explained
7 = o.p. progeny of Russian adjacent to balsam, Brooks 1(n=51)
2 = c.c. of balsam (Pb3) x balsam (poly mix) (n=29)
1 = c.c. Russian x balsam (poly mix) (n=17)
Leaf form o.p. seedlings from Russian hybrid
Leaf form of c.c. seedlings from Russian x balsam
Leaf form of c.c seedlings of balsam x balsam
11 observations hidden
Can1
71% of the variation is explained
* = coordinates of population mean of Russian x balsam (-0.922, 0.843)
* = coordinates of population mean for balsam x balsam (-1.080, -0.440)
* = coordinates of population mean for o.p. Russian (0.370, -0.012)

Table 4-7. Raw canonical coefficients for the first two canonical variables (Can1 and Can2) based on the six leaf variables measured for both the Balsam by Brooks 1 and Russian by Balsam data combinations.

Raw canonical coefficients

	Balsam by Brooks 1		Russian by Balsam	
Variable	**Can1**	**Can2**	**Can1**	**Can2**
Length	0.013	0.0008	0.019	-0.047
Width	-0.144	-0.017	0.053	-0.139
Position of maximum width	-0.017	0.032	0.107	0.219
Petiole	0.284	0.024	-0.177	0.119
Leaf base	0.038	0.320	0.018	-0.001
Leaf apice	-0.268	1.070	0.939	0.547

4.4. DISCUSSION

4.4.1. *Leaf form*

In the analysis of leaf form, a major problem was encountered and was attributed to one of the many types of heterophyllous leaves expressed in *Populus* species. One of the most common types of heterophylly, but least discussed in the literature, is the morphological difference in the development of juvenile leaves to adult leaves. In the late 1890's, four stages were proposed describing leaf development phases between juvenile and adult leaves: 1) initiation, 2) embryonic growth and the determination of form, 3) expansion, and 4) maturation (Critchfield 1960). During the 1950's, several studies suggested that the form of the juvenile leaf in poplars (and other species) is related to the duration of its embryonic stage, which can last up to five years before expansion occurs (Critchfield 1960). Morphological development of leaves from a juvenile to an adult plant was termed 'heteroblastic development' where juvenile leaves are smaller and simpler than the leaves of a mature plant (Critchfield 1960).

The leaf ratios and principal component analysis clearly distinguished between the individual points of mature trees and one-year-old seedlings, but there was no

separation between the different groups of progeny. This distinct separation between mature and juvenile plants is most likely due to heteroblastic development. The commonly used canonical variate analysis to identify different populations of *Populus* species, based on morphological leaf characteristics, was more effective in separating the progeny into different groups than with principal component analysis. However, canonical variate analysis was still not an effective tool in separating the progeny from controlled crosses (known parentage) based on the six variables used. Since juvenile leaves lack distinctive features that are commonly used in identifying poplar species, such as blade form, leaf serrations, number of teeth per blade length, and distinctive petioles (Barnes 1969; 1975; Dancik and Barnes 1975) the canonical variable analysis failed to separate the groups based on the six variables measured. However, if the seedlings were older, expressing more diagnostic features, perhaps canonical variate analysis would be a good tool to use to separate groups and possibly assess whether hybridization is occurring. For example, mature leaf blades of Brooks 1 are wavy and they have red petioles. These two characteristics could be diagnostic characters for the identification of putative hybrids of Brooks 1, although, not yet present in the progeny. In addition to using leaf characteristics, mature trees have other morphological characters that can be used in the identification of poplar species and hybrids, such as the color and shape of both reproductive and vegetative buds. In chapters two and three (data collection) it was observed that each of the hybrid poplar clones exhibited different shapes and colors of buds (Table 4-8).

Table 4-8. Observed differences in the color and shape of buds and other distinctive features in five hybrid poplar clones.

Hybrid clone	Bud color	Bud shape	Other features
Brooks 1	Medium brown	Typical bud shape found in poplars	- Wavy leaf blade and red petiole
Brooks 6	Yellow	Typical bud shape found in poplars	- Bud resin extremely sticky and yellowish in color
Russian	Yellow	Typical bud shape found in poplars	- Bud resin extremely sticky and yellowish in color
Tristis	Yellowish - brown	Slender and pointy buds compared to other poplars	
Northwest	Dark brown	Larger and plumper buds than other poplars	

1 = See Table 2-1 for parentage of hybrids.

The data reported in this study provides useful information, as the identification of naturally occurring hybrids cannot be determined using morphological leaf characteristics of young seedlings. It may be necessary to wait until seedlings are at least five-years of age when young trees are developed; however, DNA analysis could be used to determine hybrids at any age.

The use of morphological leaf characteristics of mature trees is commonly used in population studies, but there are limited studies reporting on the morphological features of one-year-old poplar seedlings. A study conducted on the tri-hybrid swarm in southern Alberta reported that no clear patterns identifying pure species of *P. balsamifera, P. angustifolia, P. deltoides,* and their naturally occurring hybrids were observed from the principal component analysis, using two-year old cuttings (Rood et al. 1986). It is believed that the leaves produced from cuttings (after two years of growth) are also juvenile, resulting from pseudo-juvenility (the rejuvenation effect of propagation), producing only late leaves, which are believed to be under limited genetic control (Rood et al. 1986).

4.4.2. *Summary and conclusion*

The objective of the research conducted in this chapter was to determine if hybridization is occurring naturally between native and non-native poplars by comparing the leaf size and shape of one-year-old open-pollinated (o.p.) seedlings with the known mother and putative fathers. From the data, I expected to see a pattern of clusters,

representing pure balsam seedlings overlapping with mature balsam, and putative hybrid seedlings lying between the known female and putative male parents. Results from the principal component analysis revealed no discernable pattern among seedlings; however, there was a clear distinction between the one-year-old progeny and the mature parents. By plotting the leaf measurements of the o.p. progeny with the progeny of similar (known) parentage (controlled crosses) observations of clusters representing putative hybrids and pure balsam progeny, overlapping with the progeny of similar parentage (controlled crosses of pure balsam and hybrid progeny) were expected. Again, the expected results from the principal component analysis did not show any pattern of clusters among the seedlings, regardless of parentage.

Canonical variable analysis was used to try and further separate the different groups of progeny (controlled and open pollinated crosses); however, this type of analysis was also ineffective in separating the groups of progeny based on the number of morphological leaf characteristics measurable on one-year-old seedlings.

The lack of discernable pattern between seedlings of known and unknown parentage is likely due to the prolonged embryonic phase of the seedlings. It is clear that the parentage of hybrid seedlings cannot be determined until seedlings are older than one-year and likely need to be approximately five-years old before morphological characteristics can be used to determine hybrids. Alternatively, DNA fingerprinting could be conducted. However, the fact that seed was collected from two female hybrid poplar clones (Russian and Walker poplar), which both exhibited high germination rates of normal seedlings does suggest that hybridization is occurring in one direction; hybrid females are receiving pollen from native balsam and/or hybrid male poplar clones. However, the success of those seeds is unknown.

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CHAPTER FIVE: General Discussion and Conclusion

5.1. *Introduction*

The primary interest in using non-native tree species worldwide has been and continues to largely be due to the belief that non-native trees are more productive than native trees (Peterken 2000; Karlman 2001). In Britain, non-native tree species were introduced as long ago as 2000 years (Peterken 2000). However, most introduced tree species, such as Norway maple in North America (Webb et al. 2001), a variety of deciduous and coniferous tree species in Britain (Peterken 2000), *Pinus contorta* in Sweden and *Pinus strobus* in France (Karlman 2001), have been widely distributed for approximately 200 to 400 years, and the introduction of non-native *Populus* species into European countries occurred as recently as 150 years ago (Stettler et al. 1996). However, within the last twenty years the installation of these non-native trees in Europe, such as *Pinus contorta*, *Pinus strobus*, and *Pseudotsuga menziesii,* have been restricted due to devastating ecological effects (Karlman 2001). As a result, research efforts are being directed to understand the vast ecological effects associated with the long-term use of non-native tree species (Karlman 2001; Webb et al. 2001; Leege and Murphy 2001; Engelmark et al. 2001; Peterken 2000). Some of the ecological risks associated with using non-native trees have been identified as the potential spread of insect pests and fungal pathogens (Knight et al. 2000; Lindelow and Bjorkman 2000), the potential spread of invasive trees into native populations, and the suppression of understory diversity (Webb et al. 2001; Ledgard 2000). Regardless of the extensive planting of non-native trees for hundreds of years in many countries, it is believed that all potential ecological effects may not be apparent yet (Peterken 2000).

In Alberta, introduced non-native poplar trees were widely planted as early as 1950 for the establishment of shelterbelts around agricultural homesteads. Even though these poplar trees have been growing in this region for as little as 50-years, these plantings of hybrid poplar clones has allowed us to take advantage of an in-situ experiment. These hybrid poplars have been flowering adjacent to native poplars in the region for nearly 40-years, providing an opportunity to gather information, regarding the potential for hybridization between native and non-native poplars. In addition, we were

also able to determine if hybrid seeds are being produced. With the Alberta Government currently changing policy regarding the deployment of non-native poplars, this research project provides information to help guide those discussions regarding the factors contributing to the potential for hybridization. In the next section, a synthesis of the information gathered from the previous chapters is summarized with a discussion of the practical implications, followed by future research suggestions and conclusions.

5.2. *Synthesis and practical implications*

The results from this study showed that hybridization could occur between *Populus (P). tremuloides* and *P. davidiana*, and between *P. balsamifera* and introduced hybrid poplars (both sexes), if all other genetic compatibilities and environmental conditions are met. However, the actual success of hybridization between native and non-native poplars may in fact be low when considering the following factors and suggestions for mitigation:

1) Dilution effect. The total amount of pollen released from our native poplar stands is profuse compared to the amount of pollen currently released from shelterbelts and potentially from larger poplar plantations; therefore, clouds of native pollen could act as a 'diluter', decreasing the concentration of hybrid pollen, and subsequent hybridization.

2) Relative distance. According to the literature, the majority of poplar pollen released can be carried up to 20 meters away from its source (Strauss 2000); therefore, the relative distance between native poplar stands and hybrid poplar plantations (for both pollen and seed dispersal) may need to be considered when establishing large poplar plantations. Furthermore, the small amount of pollen that is carried further than 20-meters away from its source is likely to be diluted with the abundant native pollen.

4) Selection of non-native poplars. The selection criteria for non-native poplars may be dependent on flowering patterns that are either earlier or later than native populations. 'Differences in flowering time' can be an important barrier in preventing hybridization (Wright 1976). Similarly, the selection of non-native poplars that flower in the early spring when spring frosts are still likely to occur may result in frozen reproductive buds, and therefore, these trees would be unable to release pollen or produce hybrid seed.

5) Physical barrier. The installation of sterile male hybrid poplars and/or native male balsam poplars (or any other fast growing species) around the perimeter of a hybrid poplar plantation may prove useful as either a physical barrier for pollen and seed movement or as a pollen diluter.

From the controlled breeding crosses, viable seed was collected from each of the four breeding combinations (balsam x balsam, balsam x hybrid, hybrid x balsam, hybrid x hybrid), indicating that native balsam and the hybrid poplar clones used in this study are genetically compatible. A seed germination test determined the proportion of normal and abnormal seedlings, and dead seed. The results showed varying proportions in each of the three categories. It appeared that the balsam x balsam mating combination produced, on average, more normal seedlings than the other hybrid mating combinations. This would initially suggest that the controlled crosses of pure balsam are, on average, more fertile in contrast to the controlled hybrid crosses, concluding that hybrid poplar clones are not as fertile as native balsam poplar. However, because hybrid seed was collected from two female hybrid poplar clones (from various locations) and four native balsam poplar clones (discussed in chapter four), and this seed exhibited high proportions of normal seedlings, I cannot conclude that hybrid poplars are any less fertile than native balsam poplars (as formerly suggested). Rather, I must infer that female hybrids observed are fertile and capable of producing normal hybrid seedlings. However, the actual establishment of hybrid seedlings has yet to be considered. Poplar seeds lack endosperm (lack of albumen found between seed-coat and embryo); therefore, seed viability ranges from four days to two weeks, depending on the environmental conditions (Moss 1938; Peterson and Peterson 1992). In this small window of opportunity, many environmental conditions need to be met for seedling establishment, such as exposed mineral soil, optimum soil moisture conditions, and optimum soil and air temperature (Peterson and Peterson 1992; Eckenwalder 1996). In Alberta, these conditions are rarely met in nature, except after a disturbance such as fire or harvesting, followed by a wet summer and on soils with good drainage (Peterson and Peterson 1992). However, even then the likelihood of seedling survival is thought to be low due to inter-specific competition with species such as *Calamagrostis canadensis* (Ken Greenway, Pers. Comm.).

The well-published use of leaf size and shape of early leaves to diagnostically characterize putative hybrids from native species was not as effective as initially believed, due to heteroblastic development in juvenile plants. Seedlings can remain in the embryonic phase (stage 2) for up to five years before leaves reach the expansion (stage 3) and maturation (stage 4) phase. Therefore, the determination of naturally occurring putative hybrids cannot be assessed morphologically until trees are approximately five years of age (length of time may vary, depending on the species), when young trees start to exhibit more definitive morphological features, such as leaf serrations, petiole color and shape, and blade shape (e.g. wavy blade).

5.3. *Future research*

By installing the seedlings from the controlled and open-pollinated crosses (used in this study) in a common garden experiment, it would be of interest to measure the leaves after five years of growth for morphological characteristics, and to determine if putative hybrids can be identified.

With regards to genetic compatibility, there is little to no information on the breeding compatibilities between the hybrid poplars used in this study and native balsam poplar; therefore, it is difficult to infer that all hybrid poplars used in this study are genetically compatible or fertile with native balsam poplar, as we only collected hybrid seed from two female hybrid poplars. Additional breeding experiments, using the hybrid poplar material (males and females) in this study and a wider selection of native balsam clones, is recommended in order to make stronger conclusions on the genetic compatibility between native and non-native poplars.

It would be of interest to identify whether hybrid seedlings have been established in Alberta, since we know that hybrid poplars in shelterbelts have been flowering for nearly 40-years, and viable hybrid seed was collected from open-pollinated female hybrid poplars. However, due to poplar's stringent seedbed requirements, it may be difficult to find established hybrid seedlings. In Alberta, optimum micro-sites for seedling establishment typically exist after a fire or after harvesting, but large-scale fires and harvesting rarely occur near agricultural communities where shelterbelt clones are currently planted. However, during the life span of these hybrid poplars (several

decades), the likelihood of hybrid seeds landing on optimum micro-sites for germination and establishment could increase.

5.4. *Conclusion*

As human consumption of wood products continues to grow, global demand for wood fiber will also continue to grow (Hartley 2002). It is believed that plantation forestry will supplement the global demand for wood fiber, as the demand for natural forests to remain intact increases (Hartley 2002). Through research and monitoring programs of non-native tree species, knowledge can be gained and shared between private land-owner's and government agencies to understand and minimize the potential ecological risks associated with the installation of non-native tree species.

This research project is the first step to understanding the potential ecological risks associated with the current use of non-native poplars. From this study, it was determined that hybridization is occurring between some native and non-native poplars based on their overlapping flowering patterns, and based on the presence of normal, open pollinated seedlings observed from two female hybrid poplar clones (from various locations in both Ft. Saskatchewan and Athabasca). However, the potential for hybridization between large poplar plantations and native populations, and successful seedling establishment can be thought of as a function of 1) dilution effect, 2) relative distance between hybrid poplar plantations and native stands, 3) the selection of male or female clones, exhibiting earlier or later flowering patterns than native populations, 4) the installation of fast growing border trees around poplar plantations, acting as a physical barrier for both pollen and seed movement or as a pollen diluter, and 5) stringent seedbed requirements. To conclude, it is important to understand the relevance of flowering phenology and the factors contributing to hybridization and seedling establishment in order to make informed decisions on the installation of hybrid poplar plantations, which will help minimize the potential risk of gene flow into our native populations.

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APPENDIX A

Table A-1. Legal land description of individual *P. davidiana* clones. Field site is located in a land race trial across from Derko's Gas Bar, south of Alberta-Pacific's (Al-Pac) mill site.

P. davidiana **Legal land description:** SW20 – 68 – 19

Clone number used in this study	Individual clone number according to Al-Pac	Field location
37	248	Rep 6, 3rd tree
38	273	Rep 8, 2nd tree
39	246	Rep 8, 1st tree
40	230	Rep 6, 4th tree
41	269	Rep 5, 3rd tree
42	269	Rep 5, 5th tree
43	246	Rep 5, 1st tree

Table A-2. Legal land description for each native and non-native poplar clones used in the Athabasca study area.

Native and non-native[1] clones (landowner's name)	Replicate[2] 1	2	Legal land description Quarter	Section	Range	Twp	Location of tree Athabasca	Al-Pac
Brooks 1 (Turkowski)	*		SW	20	68	21	*	
(Chursch)		*	NE	32	63	21	*	
Brooks 6 (Gillman)	*		NW	23	64	23	*	
Northwest (Macdonald)	*		NW	33	63	21	*	
(Guha)		*	SE	25	65	22	*	
Russian (Wheldon)	*		NE	3	67	22	*	
(Brytus)		*	NE	2	64	22	*	
Tristis (Gora)	*		NW	27	68	21	*	
Walker	*		NW	21	68	19		*
Manitou	*		NW	21	68	19		*
P. tremuloides (Aw)								
Aw 1			SE	21	68	19		*
Aw 2			SE	21	68	19		*
Aw 3			NW	21	69	19		*
Aw 4			NW	21	69	19		*
Aw 5			NW	17	68	19		*
Aw 6			NE	17	68	19		*
Aw 7			SW	5	69	19		*

Aw 8			SW	5	69	19		
Aw 9			SE	6	69	19		
Aw 10			SE	6	69	19		
P. balsamifera (Pb)								
Pb 1			SW	26	63	23	*	
Pb 2			NE	30	63	22	*	
Pb 3			SW	26	63	23	*	
Pb 4			NW	33	63	21	*	
Pb 5			NW	33	63	21	*	
Pb 6			SW	3	64	23	*	
Pb 7			SE	4	64	23	*	
Pb 8			NE	4	64	23	*	
Pb 9			NE	2	64	23	*	
Pb 10			NW	22	64	23	*	
Pb 11			NW	23	64	23	*	
Pb 12			NE	3	67	22	*	
Pb 13			SW	20	68	21	*	
Pb 14			SW	20	68	21	*	
Pb 15			NW	20	68	19		*
Pb 16			NW	20	68	19		*
Pb 17			NE	5	69	19		*
Pb 18			NE	5	69	19		*

1. Non-native clones = see parentage in Table 2-1, Chapter two.
2. Replicate = only relevant to hybrid poplar clones.

Table A-3. Legal land description for each native and non-native poplar clones used in the Ft. Saskatchewan study area.

Native and non-native[1] clones(landowner's name)	**Replicate 1**	**2**	**Legal land description Quarter**	**- Section-**	**Range-**	**Twp**
Brooks 1 (Hubert)	*		SW	4	55	23
Lamoureux		*	Lot 22-31	N/a	54	22
Brooks 6 (Paradis)	*		SW	1	55	23
(Hubert)		*	SW	4	55	23
Northwest (Hanes)	*		SW	35	54	23
Jeske)		*	SW	6	55	22
Russian (Jeske)	*		SW	6	55	22
(Daniels)		*	NW	25	56	22
P. balsamifera (Pb)						
Pb 1			NE	34	54	23
Pb 2			NW	32	54	23
Pb 3			NE	11	55	23
Pb 4			SE	13	55	23
Pb 5			SE	13	55	23
Pb 6			NE	24	55	23

Pb 7			NW	31	55	22
Pb 8			NE	31	55	22
Pb 9			NE	30	55	22
Pb 10			SE	7	55	22
Pb 11			NE	1	55	23
Pb 12[3]						
Pb 13						
Pb 14						
Pb 15						
Pb 16						
Pb 17						
Pb 18						

1. Non-native clones = see parentage in Table 2-1, Chapter two.
2. Replicate = only relevant to hybrid poplar clones.
3. Pb 12 through to Pb 18 are native male and female clones selected along the North Saskatchewan river just off Lamoureux Road and there is no land description given on the map.

APPENDIX B

Figure B-1. The average amount (percent) of pollen released and female receptivity (stage III) averaged across the three crown sections for individual ramets of Brooks 6, Brooks 1, Northwest, and Russian in both replicates 1 and 2 in Athabasca.

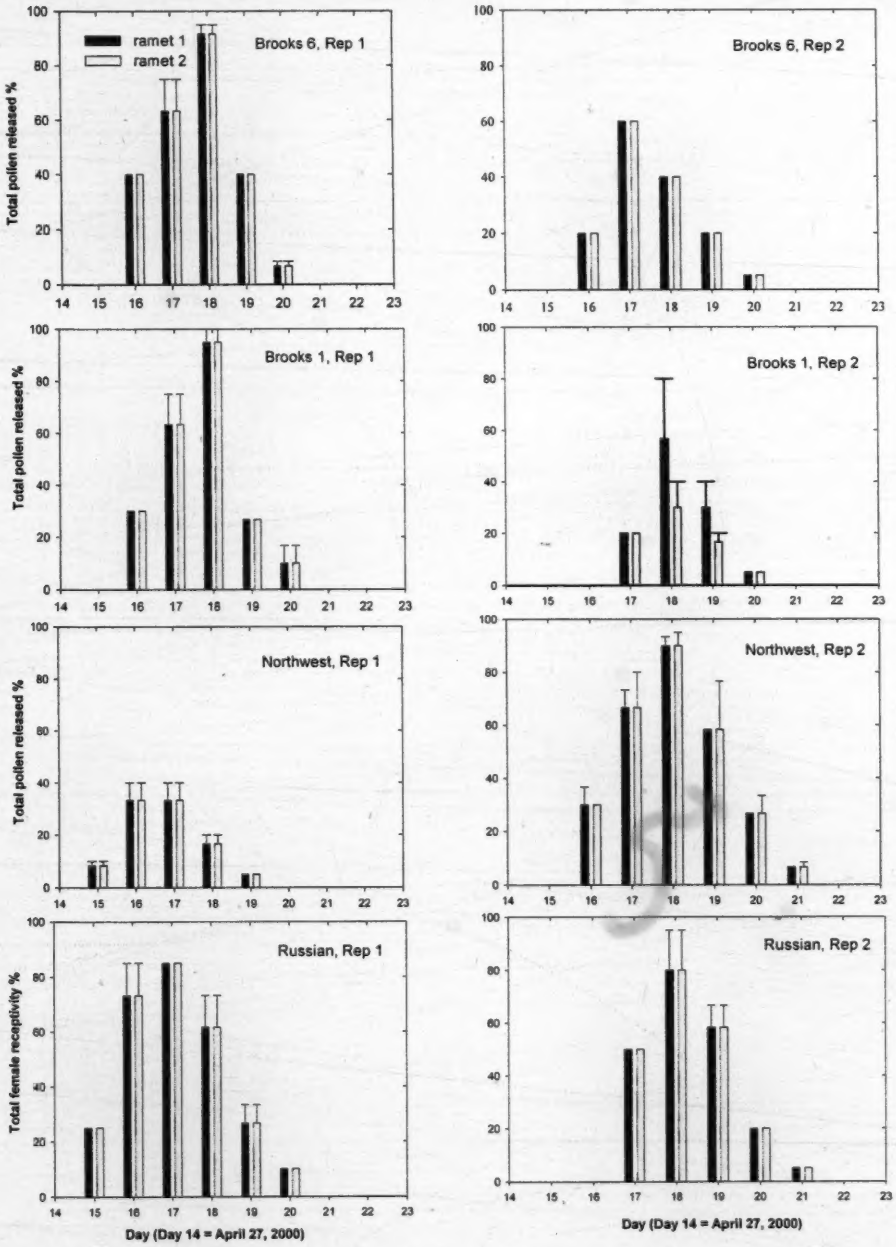
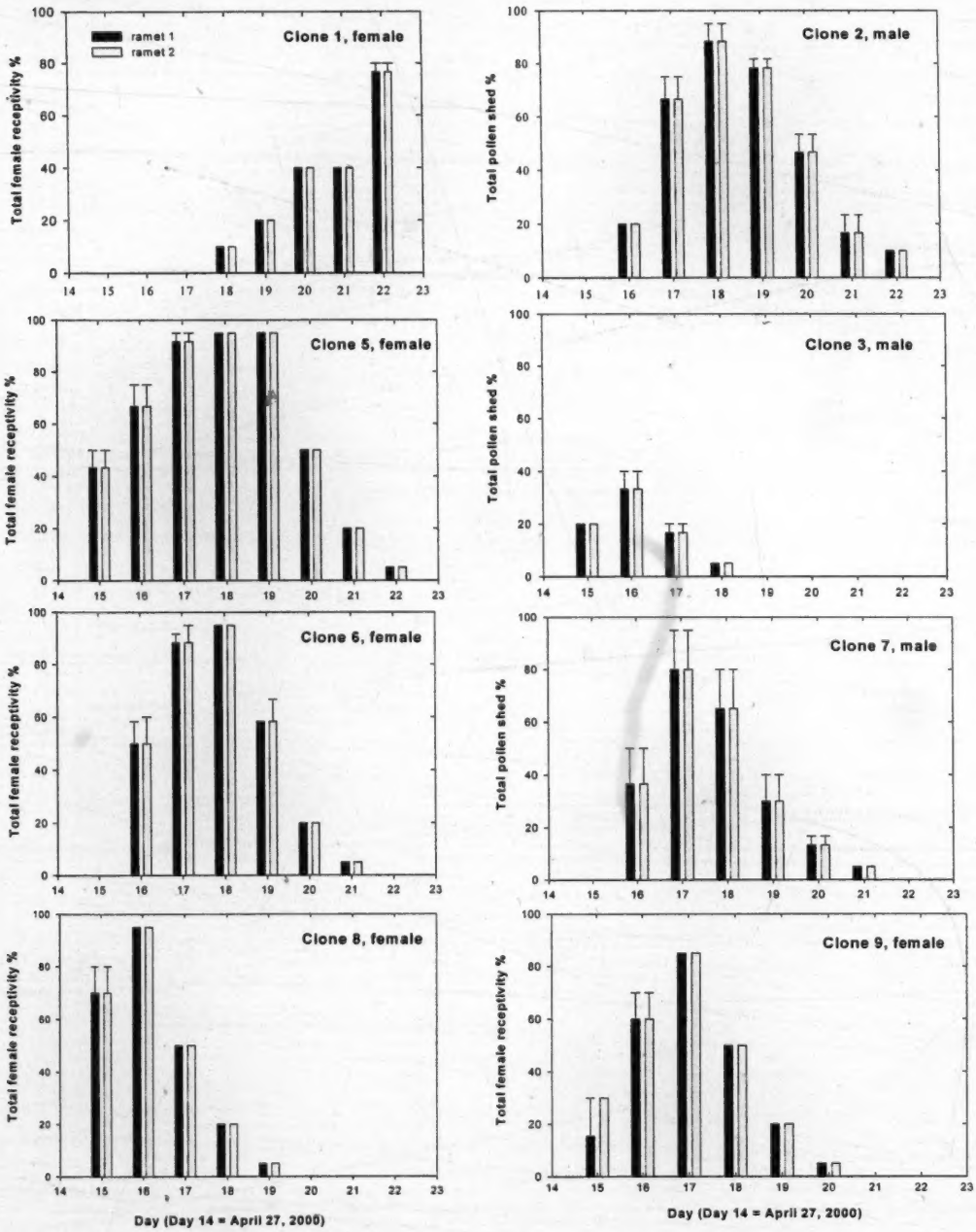
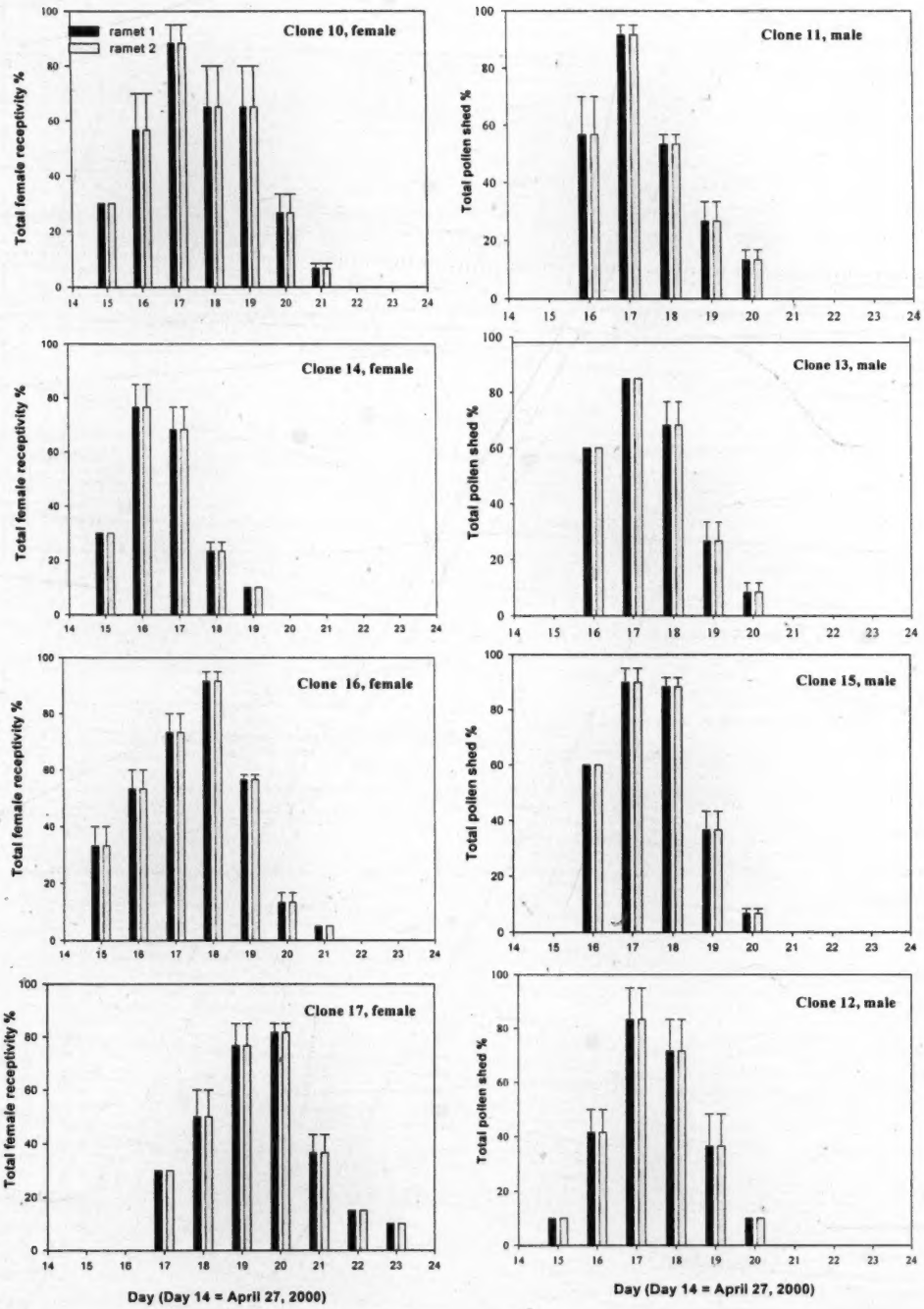


Figure B-2. The average amount (percent) of pollen released and female receptivity (stage III) averaged across the three crown sections for individual ramets of *P. balsamifera* clones (#1-18) in Athabasca.



NOTE: Clone 4 (male) released no pollen (no data collected).

continued-


ramet 1
ramet 2
Clone 10, female
Clone 11, male
Clone 14, female
Clone 13, male
Clone 16, female
Clone 15, male
Clone 17, female
Clone 12, male
Total female receptivity %
Total pollen shed %
Day (Day 14 = April 27, 2000)
Day (Day 14 = April 27, 2000)

continued....

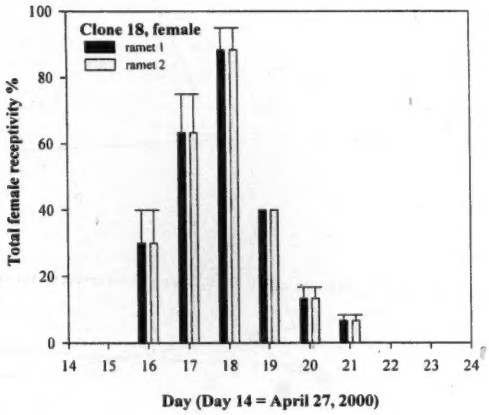

Clone 18, female
ramet 1
ramet 2
Total female receptivity %
100
80
60
40
20
0
14
15
16
17
18
19
20
21
22
23
24
Day (Day 14 = April 27, 2000)

Figure B-3. The average amount (percent) of pollen released and female receptivity (stage III) averaged across the three crown sections for individual ramets of A) Brooks 6, B) Brooks 1, C) Northwest, and D) Russian in Ft. Saskatchewan.

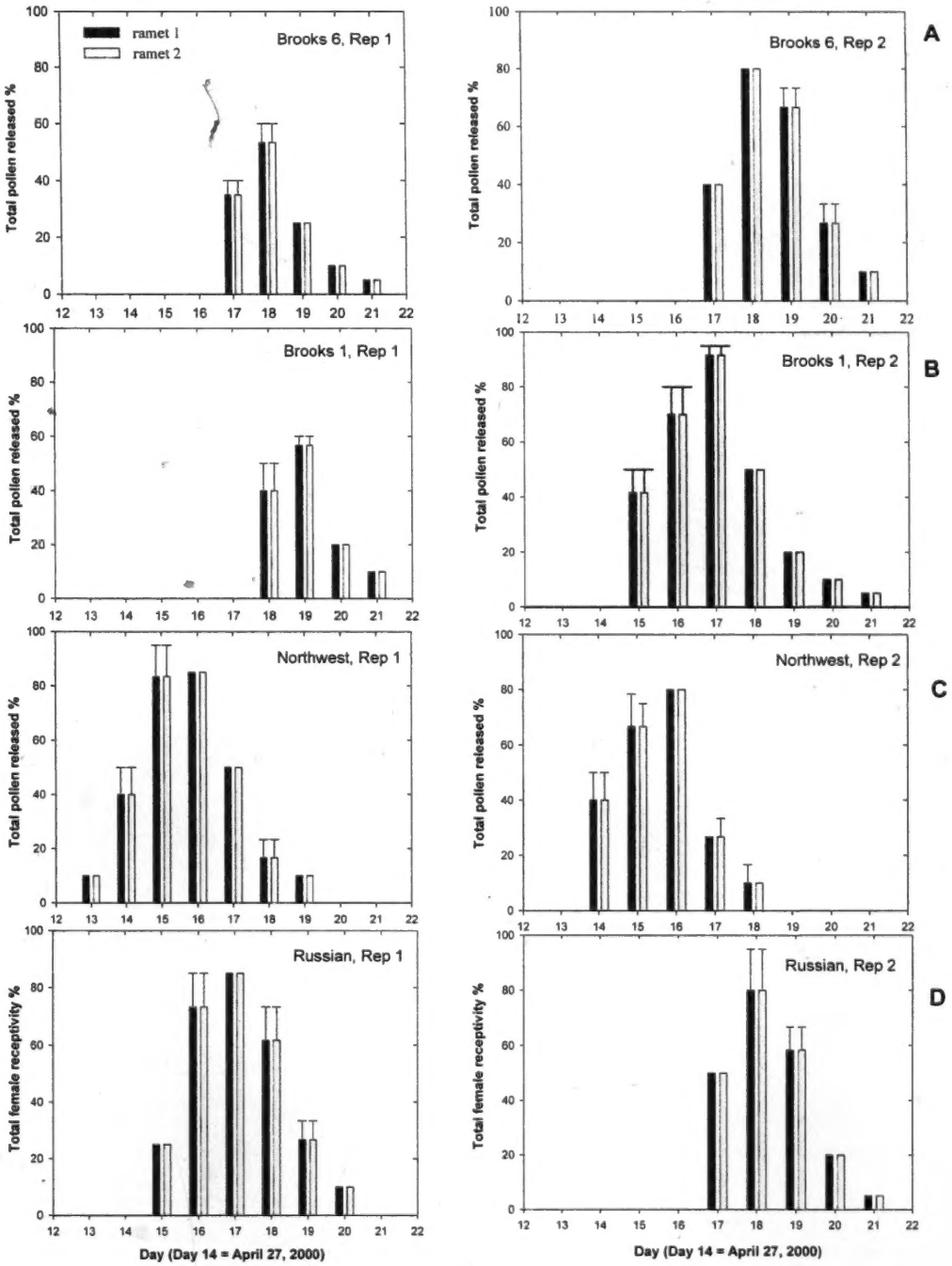
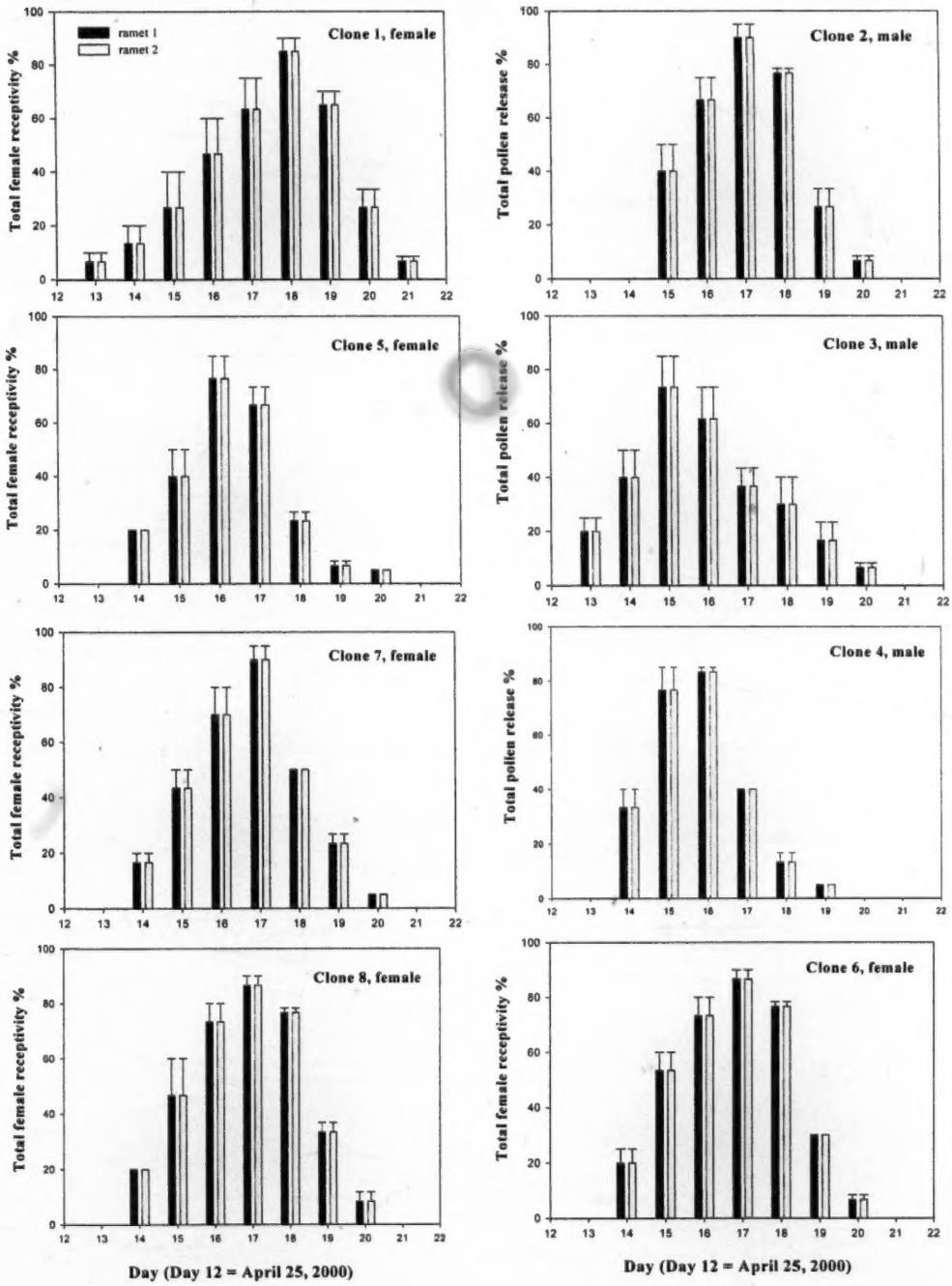


Figure B-4. The average amount (percent) of pollen released and female receptivity (stage III) averaged across the three crown sections for individual ramets of *P. balsamifera* clones (#1-20) in Fort Saskatchewan.



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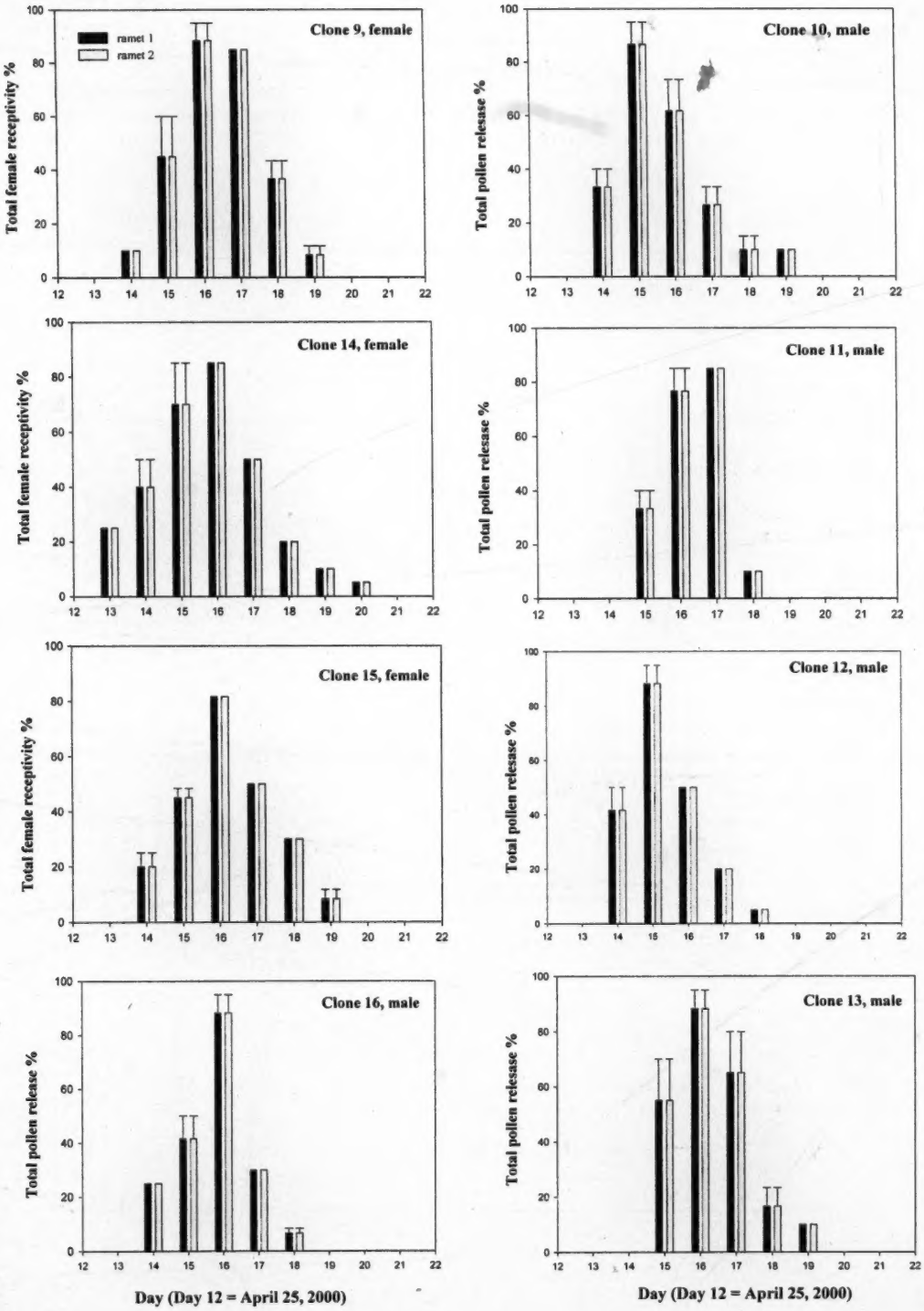
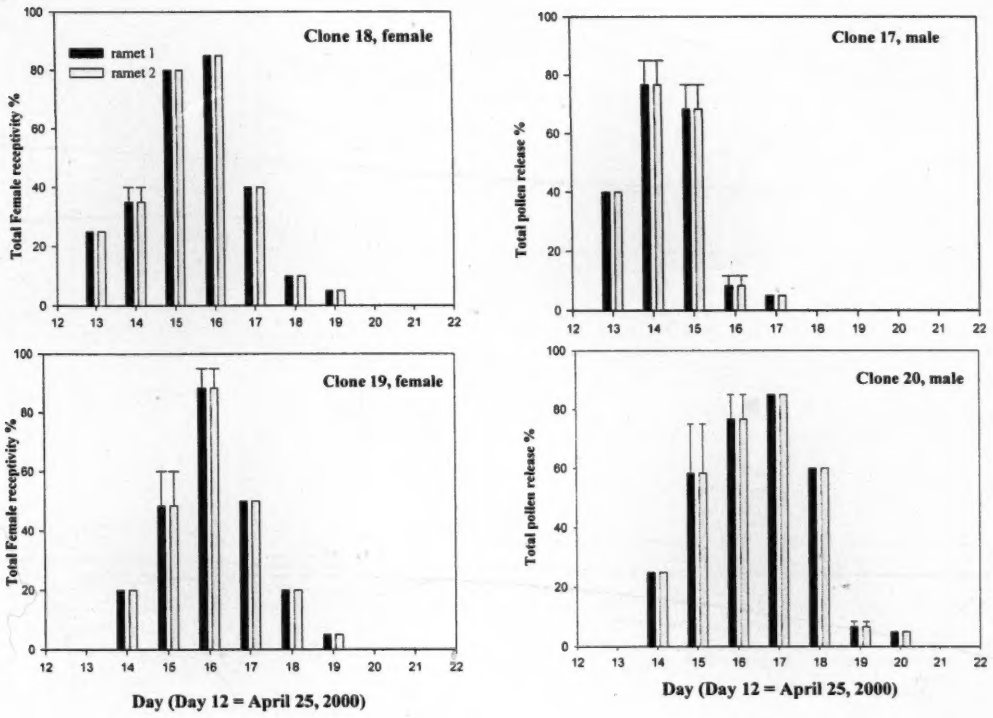

Clone 9, female
ramet 1
ramet 2
Total female receptivity %
Clone 10, male
Total pollen release %
Clone 14, female
Total female receptivity %
Clone 11, male
Total pollen release %
Clone 15, female
Total female receptivity %
Clone 12, male
Total pollen release %
Clone 16, male
Total pollen release %
Clone 13, male
Total pollen release %
Day (Day 12 = April 25, 2000)
Day (Day 12 = April 25, 2000)

continued...


Clone 18, female
ramet 1
ramet 2
Total Female receptivity %
Clone 17, male
Total pollen release %
Clone 19, female
Total Female receptivity %
Clone 20, male
Total pollen release %
Day (Day 12 = April 25, 2000)
Day (Day 12 = April 25, 2000)

APPENDIX C

Table C-1. Mating scheme, breeding cross, replicates (rep) used in the seed germination test, and the average proportion of normal and abnormal seedlings, and seed resulting in no germination (across replicates - range given).

Mating scheme	*Breeding Cross Female x Male*	*Number of rep.*	*Normal Seedling*	*Abnormal Seedlings*	*Dead Seeds*
Native[1] female x Native male	Pb2 x Pb poly-mix	3	49% (44%-52%)	41% (36%-46%)	10% (2%-19%)
	Pb3 x Pb poly-mix	3	81% (76%-84%)	18% (16%-22%)	1% (0%-2%)
Native female x Hybrid[2] male	Pb2 x Northwest	3	0%	0%	100%
	Pb2 x Tristis	3	18% (12%-24%)	33% (24%-38%)	49% (46%-52%)
	Pb2 x Brooks 1	3	84% (82%-88%)	15% (12%-18%)	1% (0%-2%)
	Pb2 x Brooks 6	3	53% (46%-56%)	39% (32%-44%)	8% (4%-14%)
	Pb2 x Manitou	1	5%	53%	42%
	Pb4 x Manitou	3	93% (90%-96%)	7% (4%-10%)	0%
Hybrid female x Native male	Russian x Pb poly mix[3]	3	32% (28%-36%)	49% (42%-64%)	19% (8%-28%)
Hybrid female x Hybrid male	Russian[1] x Brooks 1	2	21% (20%-22%)	37% (36%-38%)	42% (42%-42%)
	Russian x Brooks 6	1	20%	25%	55%
	Russian x Manitou	1	13%	37%	50%

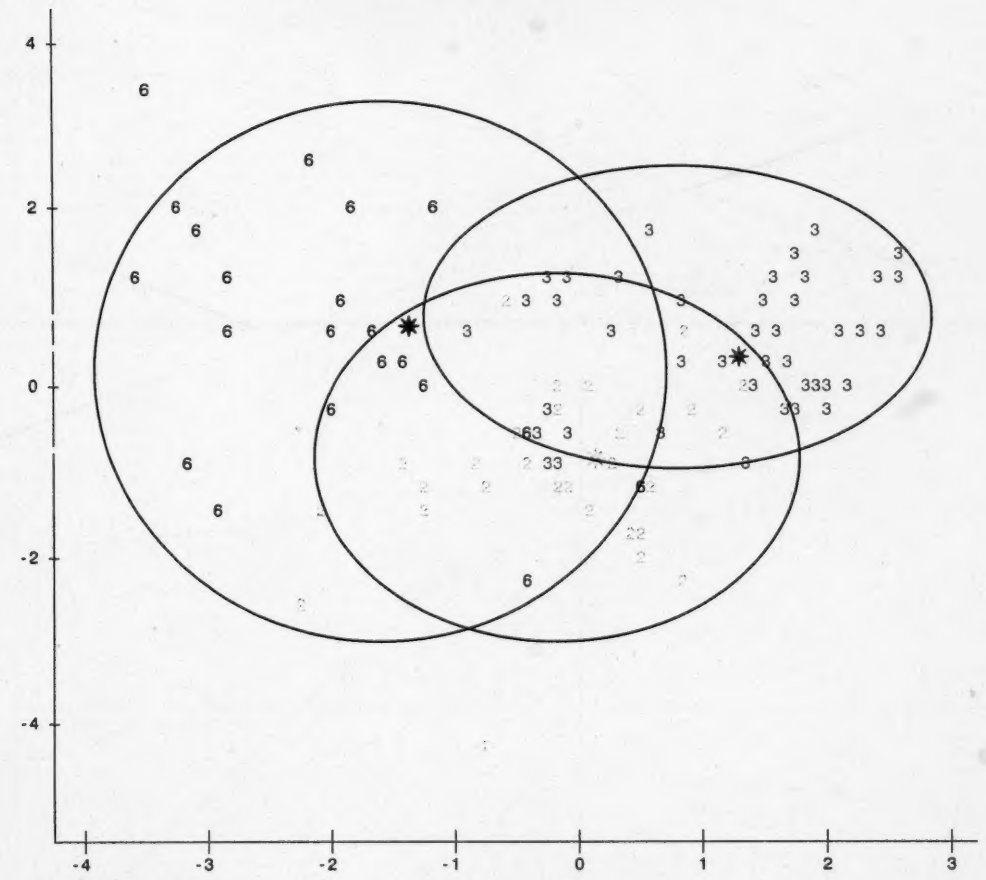
1. Native= pure species of *P. balsamifera.*
2. See Chapter II, material and methods for parentage of hybrid clones.
3. Pb poly mix = pollen mix from 4 native *P. balsamifera* clones.
4. Description of normal and abnormal seedlings are in Material and Methods section.

APPENDIX D

Figure D-1. Plot of the first and second canonical discriminate variables based on the six morphological leaf characteristics measured for open pollinated (o.p.) seedlings from native female balsam (located at Lamoureux in Ft. Saskatchewan), and controlled cross (c.c) seedlings of balsam x balsam, balsam x Brooks 1. Can 1 has the most discriminating power.

Can2 32% of the variation explained

6 = o.p. progeny of Balsam adjacent to balsam and Brooks 1 (n=42)
2 = c.c. of balsam (Pb3) x balsam (poly-mix) (n=29)
3 = c.c. balsam x Brooks 1 (poly-mix) (n=45)

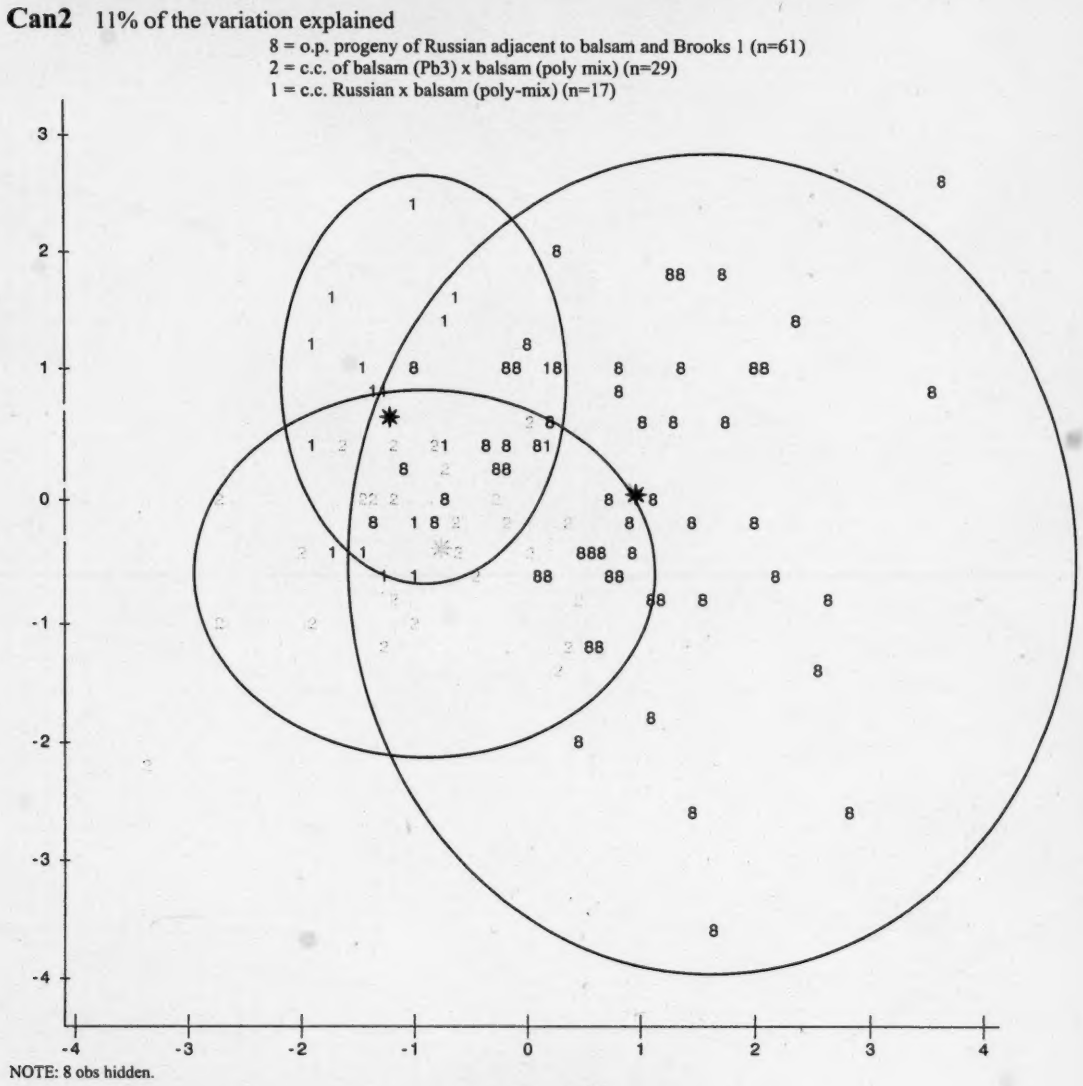


NOTE: 4 obs hidden.

Can1
68% of the variation is explained

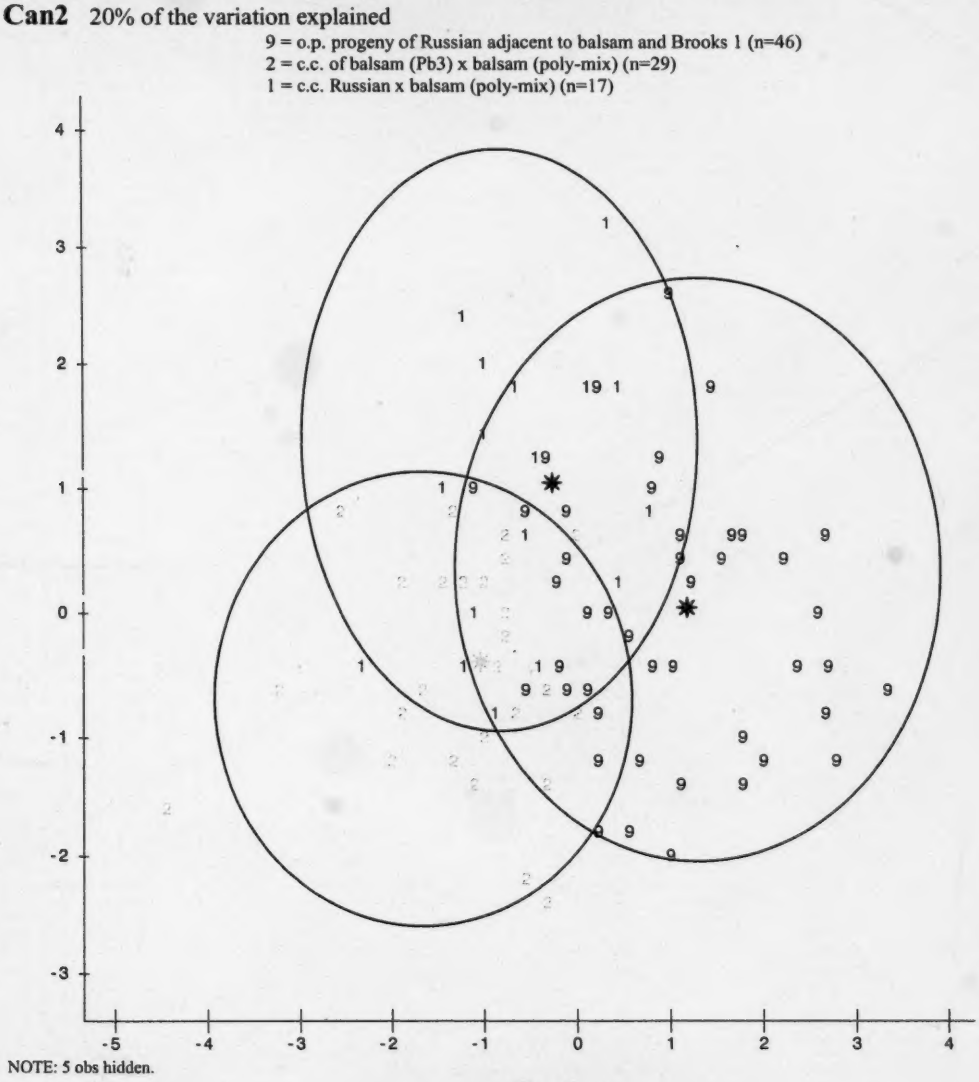
✱ = coordinates of population mean of balsam x Brooks 1 (1.033, 0.427)
✱ = coordinates of population mean for balsam x balsam (-0.088, -1.163)
✱ = coordinates of population mean for o.p. balsam (-1.570, 0.519)

Figure D-2. Plot of the first and second canonical discriminate variables based on the six morphological leaf characteristics measured for open pollinated (o.p.) seedlings from native female Russian (located at Brytus in Athabasca), and controlled cross (c.c) seedlings from balsam x balsam, Russian x balsam. Can 1 has the most discriminating power.


Can2 11% of the variation explained
8 = o.p. progeny of Russian adjacent to balsam and Brooks 1 (n=61)
2 = c.c. of balsam (Pb3) x balsam (poly mix) (n=29)
1 = c.c. Russian x balsam (poly-mix) (n=17)
NOTE: 8 obs hidden.
Can1
89% of the variation is explained

✸ = coordinates of population mean of Russian x balsam (-1.101, -0.634)
✸ = coordinates of population mean for balsam x balsam (-1.008, -0.391)
✸ = coordinates of population mean for o.p. Russian (0.786, 0.009)

Figure D-3. Plot of the first and second canonical discriminate variables based on the six morphological leaf characteristics measured for the open pollinated (o.p.) seedlings from native female Russian (located at Shymoniak in Athabasca), and controlled cross (c.c) seedlings of balsam x balsam, Russian x balsam. Can 1 has the most discriminating power.



✱ = coordinates of population mean of Russian x balsam (-0.604, 0.955)
= coordinates of population mean for balsam x balsam (-1.139, -0.416)
✱ = coordinates of population mean for o.p. Russian (0.941, -0.091)